A STUDY ON VENTILATOR ASSOCIATED PNEUMONIA WITH SPECIAL REFERENCE TO MULTIDRUG RESISTANT PATHOGENS IN A TERTIARY CARE HOSPITAL.

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CERTIFICATE

This is to certify that this dissertation titled "**A STUDY ON VENTILATOR ASSOCIATED PNEUMONIA WITH SPECIAL REFERENCE TO MULTIDRUG RESISTANT PATHOGENS IN A TERTIARY CARE HOSPITAL."** is a bonafide record of work done by **DR. K.VASANTHI,** during the period of her Post Graduate study from 2013 to 2016 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 600003, in partial fulfillment of the requirement of **M.DMICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.RMedical University to be held in April 2016.

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DECLARATION

I declare that the dissertation entitled " **A STUDY ON VENTILATOR ASSOCIATED PNEUMONIA WITH SPECIAL REFERENCE TO MULTIDRUG RESISTANT PATHOGENS IN A TERTIARY CARE HOSPITAL.**" submitted by me for the degree of M.D. is the record work carried out by me during the period of **October 2014–August 2015** under the guidance of **Dr. R.Vanaja M.D.,** Professor, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2016.

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INTRODUCTION.

"Ventilator-associated pneumonia" (VAP) is defined as 'pneumonia' that occurs more than 48 hrs following endotracheal intubation' and initiation of "mechanical ventilation".It is characterized by the presence of a new or progressive "radiographic infiltrate", fever', altered 'white blood cell count',changes in "sputum characteristics" and detection of a 'causative pathogen'.(1)

VAP is the frequent ICU acquired infection among patients on Mechanical Ventilation.(1)

VAP is a subgroup of Hospital Acquired Pneumonia.It occurs in 0.27% of

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DISSERTATION TITLE: A study on ventilator associated pneumonia with special reference to multidrug resistant pathogens in a tertiary care hospital.

ABSTRACT :

Background:

Ventilator Associated Pneumonia (VAP) is the most frequent intensive care unit (ICU) acquired infection. The aetiology of VAP varies with different patient populations and types of ICUs.

Methodology:

Endotracheal aspirates/bronchioalveolar lavage were collected from patients on mechanical ventilation for > 48hrs and processed quantitatively to determine the various aetiological agents causing VAP and the prevalence of multidrug resistant (MDR) pathogens.Combination disc method, Modified Hodge test, EDTA Combined disc test and AmpC disc test were performed for the detection of extended spectrum beta lactamases (ESBL), carbapenemases, metallo betalactamases (MBL)and AmpC β lactamases respectively.

Results:

The incidence of VAP was 16 per 1000 ventilator days.In this study,34.8% of the cases were early onset VAP,while 65.2% were late onset VAP.

Klebsiella pneumonia,Klebsiella oxytoca and Pseudomonas aeruginosa were more common in early onset VAP, while non fermenters (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*)were predominantly associated with late onset VAP.70% of the isolated VAP pathogens were multidrug resistant. ESBL was produced by 100% Escherichia coli, 67% of *Klebsiella pneumonia* ,100% of *Klebsiella oxytoca*

respectively. MBL was produced by 33% of *P. aeruginosa* and 33% of *Acinetobacter baumannii*.AmpC betalactamases were produced by 17% of *Pseudomonas* aeruginosa,22% of *Acinetobacter baumannii* and 33% of *Klebsiella pneumonia*.Of the *S. aureus* isolates, 100% were methicillin resistant. Prior antibiotic therapy,reintubation,Emergency intubation and hospitalization of five days or more were common risk factors associated with VAP.

Conclusions:

VAP is increasingly associated with MDR pathogens. Production of ESBL, AmpC betalactamases and metallo betalactamases were responsible for the multidrug resistance of these pathogens. Increasing prevalence of MDR pathogens in patients with late onset VAP indicate that appropriate broad spectrum antibiotics should be used to treat them.It is useful in implementing simple and effective preventive measures including precaution during emergency intubation, minimizing the occurrence of reintubation, and judicious use of antibiotics.

Key words: Ventilator Associated pneumonia,Intensive care unit,Extended spectrum beta lactamase,AmpC beta lactamase,Metallobetalactamase.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is defined as pneumonia that occurs more than 48 hrs following endotracheal intubation and initiation of mechanical ventilation.It is characterized by the presence of a new or progressive radiographic infiltrate, fever, altered white blood cell count,changes in sputum characteristics and detection of a causative pathogen.(1)

VAP is the frequent ICU acquired infection among patients on Mechanical Ventilation.(1)VAP is a subgroup of Hospital Acquired Pneumonia.It occurs in 9-27% of patients on ventilator.(2)India has an overall crude mortality of 67.4% in patients with pneumoniain ICU, with 40% of the mortality is attributable to infection alone.(6)

VAP is usually categorised as early onset and late onset VAP.Early onset VAP occurs within first 4 days of mechanical ventilation (MV),usually carries a good prognosis and are likely to be due to organisms sensitive to antibiotics. Late onsetVAPdevelops five (or more) days after initiation of Mechanical ventilation.It is caused by MDRpathogens and is associated with increase in patient mortality and morbidity (3).

The risk of VAP is highest early in the course of hospital stay, and is estimated to be 3% per day during the first 5days of ventilation, 2% per day during 5 -10 days of Mechanical ventilation and 1%per day after 10 days.(2)VAP needs to be diagnosed earlyand treated with appropriate antibiotics as reported by differentstudies,which showed that delayed administration of antibiotic therapy that is appropriate has been associated with increased mortalityin patients with VAP.(2,3)

The diagnosis of pneumonia in mechanically ventilated patients is based on the combination of clinical, radiological and microbiological criteria.(7)The lower respiratorytract samples obtained either by bronchoscopic(eg.BAL,PSB)ornonbronchoscopic methods(Endotracheal aspirates) are used in the diagnosis of VAP.The endotracheal aspirates are easy to collect and have a high sensitivity.(5)

The common pathogens causing VAP include*Pseudomonas aeruginosa*, *Acinetobacterspecies*, *Klebsiellapneumoniae*, *Enterobacterspecies,* and MRSA (*methicillin Resistant Staphylococcus aureus*).Among them *Pseudomonas* and *Acinetobacter species* are often multidrug resistant which is attributed to the production of ESBL(Extended spectrum beta lactamases),Amp C beta lactamases and metallo beta lactamases.(2)

Thus ,VAP poses grave complications in endotracheally intubated patients in ICU's worldwide.It leads to adverse clinical outcomes and increase in healthcare costs.(1)

The causes of VAP are different among different patient populations and also in different type of Intensive care units.Hence the local microbial flora associated with VAP and their sensitivity pattern should be studied in all clinical setting which may guide in the effective and rational utilization of antimicrobial agents.Our Institution is a tertiary care hospital providing critical carefacilities,where many patients routinely undergo assisted mechanical ventilation.

The present study isundertaken to detect bacterial and fungal etiological agents commonly associated with VAP in our hospital Intensive care unit and also to

study their antibiotic susceptibility patterns with specialemphasis on multidrug resistant pathogens.

AIMS AND OBJECTIVES

Aims:

1. To identify the bacterial and fungal etiological agents associated with Ventilator Associated Pneumonia(VAP).
2. To evaluate the antimicrobial susceptibility pattern for the isolates.
3. To determine the frequency of Multidrug resistant (MDR) pathogens among the VAP patients.
4. Toanalyse the risk factors associated with VAP.
5. To calculate the Ventilator associated pneumonia rate per 1000 ventilator days.
6. Toasses the clinical outcome in VAP patients.

Objectives:

1. To monitor adult patients on mechanical ventilator in Medical Intensive care unit for the development of VAP by clinical and radiological criteria
2. To Process endotracheal aspirates and BAL samples quantitativelyfor the identification of causative organism.
3. To detect the presence of ESBL, AmpC beta lactamase, and (MBL) Metallobetalactamase production among the MDR pathogens.
4. Toanalyse the risk factors for the development of Ventilator Associated Pneumoniaby clinical history and medical records.

5. To monitor the total number of patientson mechanical ventilator and the totalventilator days exposed by the patients inMICU.
6. To follow up the patients with VAP for prognosis.

REVIEW OF LITERATURE.

History:

The Roman physician Galen may have been the first to describemechanical ventilation: "If you take a dead animal and blow air through its larynx [through a reed], you will fill its bronchi and watch its lungs attain the greatest distention.(11).Vesalius too describes ventilation by inserting a reed or cane into the trachea of animals**(10)**.

The iron lung, also known as the Drinker and Shaw tank, was developedin 1929 and was one of the first negative-pressure machines used for long-term ventilation. It was refined and used in the 20th century largely as a result of the polio epidemic that struck the world in the 1940s. The machine is effectively a large elongated tank, which encases the patient up to the neck. The neck is sealed with a rubber gasket so that the patient's face (and airway) are exposed to the room air**.(11)**

The design of the modern positive-pressure ventilators were mainly based on technical developments by the military during World War II to supply oxygen to fighter pilots in high altitude. Such ventilators replaced the iron lungs as safe endotracheal tubes with high volume/low pressure cuffs were developed.The popularity of positive-pressure ventilators rose during the polio epidemic in the 1950s in Scandinavia and the United States and was the beginning of modern ventilation therapy**(11)**

Definition:

The exact definition of VAP is still a matter of debate ,because of the lack of criteria which is able to distinguish itfrom other pulmonary conditions in patients who are critically ill.

In 2005, the American Thoracic Society and InfectiousDiseases Society of America jointly published practicalguidelines on hospital-acquired infection which defines(HAP)Hospital acquired pneumonia as pneumonia that occurs 48 hours or more after admission, which was not incubating at the time of admission whereas VAP isdefined as pneumonia that arises more than 48-72 hrs after endotracheal intubation.(2)The 48-hours time frame was set to differentiate any newinfection from processes already ongoing at the moment ofintubation. VAP is categorised into an early and late onsetVAP,due to the difference in epidemiological features and treatment options available for the two forms (2).

Similarly, the Centers for Disease Control (CDC) defined VAP as pneumonia that occurs in a patient who was intubated and ventilated at the time of or within 48 hrs before the onset of the pneumonia.The importantdifference in the above two definition is that in CDC,diagnostic criteria does not require a window of time after intubation to be called as VAP.(13)This difference is relevant, because the CDC's definition includes pneumonia occurring within the first 2 days of mechanical ventilation,whichwould be excluded using the American

ThoracicSociety/Infectious Diseases Society of America definition,leading to an increase in VAP incidence.

Pneumonia acquired within 48 hours after hospital admission as aconsequence of emergency intubation, aspiration due to decreased level ofconsciousness and coma, or cardiopulmonary resuscitation are excluded fromdefinition of VAP .(2)

Categorisation of VAP:(3)

Early-onset VAP

Ventilator associated pneumonia occurring in the first four days ofendotracheal intubation and initiation of mechanical ventilation is called as early onset VAP anditaccounts for a better prognosis.

Late-onset VAP

Ventilator associated pneumonia developing after four days of mechanicalVentilation is defined as late onset VAP andis associated with higher mortality and is often caused by multidrug resistant bacteria.(2)

Incidence:

VAP is one of the most important nosocomial infections in ICU causingsignificant morbidity and mortality.(12)Accurate data on the epidemiology of VAP arelimited by the lack of standardized criteria for its diagnosis.The incidence of VAP is different among various studies which depends on the definition used to diagnose VAP,the hospital or ICUtype,the study population and the level of exposure to antibiotics.The VAP rate is higher in surgical ICUs when compared to medical ICU's(13,17).

A study was conducted with Athenians ,which identified patients at a greater risk of developing VAPand the incidence of VAP among patients on mechanical ventilator was found to be around eight percent.

(12)Bowton DL et al(24) (2006) showed in their study that the nosocomial pneumonia rate in mechanically ventilated patients varies from 9% to 68% andmortality rates varies from 33% to 71%.

Wagh H andAcharya D stated the rates of VAP to range from 9 to 27% with the presence ofMDR pathogens and associated with high morbidity and mortality rates.VAP also increased length of ICU stay by 28%.(2)

A study from India evaluated 51 patients in the critical care unit and found a mortalityrate of 37% attributable to VAP, which also correlated very well with higher APACHEIII scores; 33% of the cases were early onset, and 67% were late onset.Themortalityrates of patients with or without VAP in different studies were ; 71% and 28% (20) 55% and 25%,(19)and 33% and 19% (21) respectively.

There is increased risk of acquiring pneumonia when there is increase in duration of mechanical ventilation.(23). The prognosis for VAP caused by Gram negative aerobic bacilli is considerablyworse than that for infection with Gram positive pathogens.According to Study by Fagon et al(20) mortality associated with*Pseudomonas or Acinetobacter* pneumonia was 87% compared to 55% for pneumoniadue to other organisms.

Similarly,(Kollef and coworkers)(17) demonstrated that patientswith VAP due to pathogens like *Acinetobacter spp. ,Pseudomonas aeruginosa,* had a significantly

higher mortality rate(65%) when compared to patients with VAP due to other organisms (31%).

Risk factors for VAP

During the first four days of artificialventilation , insertion of multiple central venous line,emergency intubation and intravenous sedatives were found to be independent risk factors of Ventilator Associated Pneumonia,whereas after 4 days of mechanical ventilation the risk factors of VAP are tracheostomy, reintubation and treatment with H2 receptor antagonists.(12,15)

Host Factors:

Surgery:

Post surgical patients are at increased risk for the development of VAP(4).Risk for VAP differs among various types of surgicalICU's.Cardiothoracicsurgery and trauma patients were more prone to develop VAP.(4)

Burns:

VAP is more common in serious thermal injury patients,especially if there is co-existent inhalation injury or if the patient is intoxicated at the time of admission.(30)

Sinusitis:

Patients with infectious sinusitis are at high risk for VAP,with 67% of them developing pulmonary infection shortly following the diagnosis of sinusitis.(4).

Immunosuppression:

Patients who areimmunosuppressedfrequently develop pulmonary infectious complications leading to respiratory failure,which necessitate mechanical ventilation.Immunocompromised patients are at risk fordeveloping VAP due to opportunistic microbes as well as the common pathogens.(30)

Intervention factors:

Intubation:

Intubation is the most important risk factor associated with a 3 to 21 fold risk for developing VAP.It increases the risk by:

1.causing trauma to nasopharynx or oropharynx

2.impairing swallowing of secretions

3.Increasing bacteriological adherence and colonization

4.causing ischemia secondary to cuff pressure

5.impairingciliary clearance and cough

6.causing pooling of contaminated secretions and leakage of secretions around the cuff.

7.requiring frequent suctioning.(31)

Tracheostomy and reintubation:

Aspiration during reintubation and the presence of tracheostomy may contribute to the development of VAP.(29,34)

Nasal intubation:

Nasal intubation increases the risk of VAP by blocking the nasal ostia and promoting the development of sinusitis which act as a source of VAP pathogen.(34)

Duration of mechanical ventilation:

The incidence of VAP increases with duration of mechanical ventilation.The risk of VAP is highest early in the course of hospital stay and is estimated to be 3% per day in the first week of MV,2% per day in the second week and 1% per day later(32).As mechanical ventilation is most often short term,about half of all episodes of VAP are of early onset type(4).

Nasogastric tube:

Nasogastric tube may increase oropharyngeal colonization and cause stagnation of secretions.It also increases gastro-esophageal reflux and hence the risk of aspiration.(4,33)

Supine position:

Seriously ill patients who spend greater time at backrest elevations of less than 30^0 during the first day of intubation are more prone to develop VAP.(34)

Antacids:

Patients receiving H_2receptor antagonists were at high risk for developing VAP(4).

Prior antibiotic therapy:

Prior antibiotic therapy appears to have an interesting dual effect.Thoughthe use of antibiotics prophylactically reduces the risk of early onset VAP (due to antibiotic susceptible bacteria),it may predispose to late onset VAPdue to colonization and infection with multi drug resistant pathogens.(4,31,30)

Respiratory equipment:

Respiratory equipment itself may act as a source for bacteria responsible for VAP. In 104 mechanically ventilated patients,Closed versus open suctioning systems were

compared and VAP rate was found to be lowerin patients treated with the closed system when compared with those patients with the open system .(35,36)

Mechanical ventilators with humidifying cascades have high levels of tubing colonization as well as condensate formation that may be a risk factors for pneumonia.(37)

As most of the tubingcolonization was derived fromsecretions of the patients, the highest bacterial counts wereseen near the endotracheal tube.

The use of heat-moisture exchangers (HMEs) has been studied by various studies in placeof conventional heated-water humidification systems.

HMEs are associated with lower incidence of VAP than heated humidifiers(38).(Lorente L et al)(39) suggests that using HMEs instead of heated humidifiers, may increase the VAP rate.

Kollef MH et al (40) suggested improper hand washing results in crosscontamination of patients which is the major personnel related risk factor for VAP. Patientswho are in mechanical ventilation often need interventions such assuctioning of secretions or manipulation of the ventilator circuit.

Failure inadherence to proper hand washing techniques and not changing the gloves while handling manycontaminated patients has been associated with an increase in VAP rate.

Etiological agents:

Microorganisms causing VAP may differ according to the specificdiagnostic methods used ,populationunder study, the durations of stay in hospital and ICU's.

The type of organism causing VAP usually depends on mechanical ventilationduration.Early onset VAP is caused by antibiotic sensitive pathogens, whereas late onset VAP is caused by multi drug resistant pathogens.

Bacteria causing early onset VAP are*Hemophilusinfluenzae, Streptococcus pneumoniae,methicillin-sensitiveStaphylococcusaureus*(MSSA),*Escherichia coli,Proteusspecies,Klebsiellapneumoniae,Enterobacter species,and Serratiamarcescens.*

MDR bacteriasuch as*methicillin-resistant S. aureus(MRSA), Acinetobacter sp., Pseudomonas aeruginosa, and* extended-spectrum beta-lactamase producing bacteria (ESBL) are typically pathogens of late onset VAP (33)

Oropharyngeal commensals like*Streptococcus viridans, Corynebacterium, coagulase negative staphylococcus (CONS) and Neisseria*species can attain clinically significant numbers in the lower airways.

*Candida albicans*and other *Candida*species can be isolated commonly from endotracheal aspirates, but usually it representscolonization of the airways, rather

than pneumonia inimmunocompetentpatients.It rarely requires antifungal therapy.(2)

Multidrug resistant pathogens:

The pathogens causing ventilator associated pneumonia like *Acenetobactersp,pseudomonassp*,(nonfermentors) and other GNB‘s producing ESBL, AmpCbetalactamases, display high levels of resistance to antibiotics.These bacteria are called as multidrug resistant pathogens.(3)

Pathogens causing VAP, their frequency and their possible mode of multi drug resistance, if any, are listed below (2)–(4):

1. *Pseudomonasspecies*(24.4 %): (Upregulation of efflux pumps, decreased expression of outer membrane porinchannel, acquisition of plasmid mediated metallobetalactamases).

2.*Staphylococcusaureus*(20.4 %, of which > 50 % MRSA): Production of a penicillin-binding protein (PBP) with reduced affinity for beta-lactam antibiotics. Encoded by the mecA gene.

3. Enterobacteriaceae (14.1 % – includes *Klebsiella spp., E. coli, Proteus spp.,Enterobacter spp., Serratia spp.,* Citrobacter *spp*.): Plasmid mediated production of ESBLs, plasmid mediated AmpC-type enzyme.

4. *Streptococcusspecies* (12.1 %).

5. *Hemophilusspecies* (9.8 %).

6.*Acinetobacterspecies*(7.9 %): Production of metalloenzymes or carbapenemases.

7. *Neisseriaspecies* (2.6 %).

8. *Stenotrophomonasmaltophilia*(1.7 %).

9. *Coagulase-negative staphylococcus* (1.4 %).

10. Others (4.7 % – includes *Corynebacterium, Moraxella, Enterococcus*, fungi).

Pathophysiology:

VAP occurs by four main routes:

1.Aspiration of infectious secretions,either directly from the oropharynx or secondarily,by reflux from the stomach,

2.Inhalation of contaminated air or infectious aerosols

3.The development of biofilm acts as a bacterial reservoir for inoculum in to lung.

4.Hematogenous spread of microbes to the lung from a distant focus of infection.

The inspired air is filtered and humidified in the upper airways.The presence of antimicrobial agents in saliva ,an intact mucociliary clearance and cough reflex acts as a normal defence mechanism in the host to prevent invasion of bacteria.In ICU patients who are critically ill,these defences are altered which favours the pathogens to reach the distal lung and multiply to cause an invasive disease.(4).

A well structured biofilm develops rapidly within hours of tracheal intubation.Bacteria easily attach to the polyvinychloride (PVC) surface of the ETT, where they multiplyand differentiate their phenotype within the extracellular self-produced matrix (8).

The most common organisms which are associated with biofilm formation are Gram negative bacterialand fungal organisms. The organisms can colonize the

endotracheal tube atthe moment of intubation due to leakage of secretions outside the cuff, or following Endo tracheal suctioning.

There is increased bacterial resistance to antimicrobial agents due to biofilm formation, which is probably related todifferent cellular and extracellular mechanisms.

Biofilm can act as a reservoir for highly infectivemicroorganisms that can detach themselves and enter the lungs as aconsequence of endotracheal aspiration or inspiratory flow during mechanical ventilation.(8).

Diagnosis:

Clinical diagnosis:

The clinical diagnosis of VAP is made when a radiographic infiltratethat is progressive or new plus at least 2 of the following 3parameters-leukocytosis, orfever or purulent tracheal secretions.

An alternative approach to diagnose VAP clinically is suggested byPugin et al.,based on fever, leukocyte count, purulent tracheal secretions,difference in oxygenation, radiographic changes, Gram stain and culture results is calculation of Clinical Pulmonary Infection Score (CPIS).(37,38).

A CPIS of more than 6 was associated with a clinical definition of pneumonia which has 93% sensitivity and 100% specificity compared withquantitative BAL culture.

Modified CPIS is suggested by Singh et al. who used first five clinical variables to diagnose VAP initially, then after 72 h recalculate CPIS based on all the six clinical

variables,whichhelps to stop antibiotics in those patients with a (CPIS<6)a low scorepersistently after three days of treatment.(39).

Fartoukh et al(2) suggested that Gram stain results should be incorporatedinto the score which increase CPIS sensitivity.

The Clinical Pulmonory Infection Score (CPIS) Ref(1)		
Assessed Parameter	**Result**	**Score**
Temperature	≥38.5°C & ≤ 38.9°C	Point 1
	>39°C or <36°C	Point 2
Blood leucocyte count (cells/mm^3)	<4000 or >11000.	Point 1
	+ >50% band forms	Point 2
Oxygenation(mmHg) Pa02/Fio2	<240 and no ARDS	Point 2
Chest X-ray	No infiltrates	Point 0
	Patchy or diffuse infiltrates	Point 1
	Localised infiltrates	Point 2
Tracheal secretions (subjective visual scale)	Mild/non purulent	Point 1
	Purulent	Point 2
Culture & Gram stain of endotracheal aspirate.	Moderate or heavy growth	Point 1
	Same morphology on Gram stain	Point 2

Radiological diagnosis:

The findings in chest radiograph(eg.progressiveinfiltrate,rapidcavitation,single air bronchogram etc.) were associated with a specificity of 96% for diagnosis of VAP. Specific findings in radiograph are relatively uncommon, help in excluding the VAP diagnosis,when it is normal(7).

Other conditions like emphysema, chemical pneumonitis,cardiopulmonaryedema,drug reaction, pulmonary contusion,atelectasis etc. may show similar radiographic abnormalities consistent with VAP.(7,4,33)

Laboratory diagnosis:

The microbiological diagnosis is based on microscopy and culture of secretions obtained from the lower respiratory tract(41) as suggested below.

1)The samples should be collected preferably before starting antibiotics.

2) Adequate amount of sample is essential.

3) Specimenprocessing within 30 min is ideal,otherwiserefrigerated in case of delay of few hours.(4,41,42).

Microscopy:

The DirectGramstain is used to detect bacteria as well as yeast cells insamples from respiratory tract.The presence of greater than 10 squamous epithelial cells per low power field in gram stain is used to reject the endotracheal aspirate sample from processing.The number of pus cells is generally not indicative of a good specimen in patients with VAP(43,44).

The presenceof pus cells is not specific for a culture to be positive,but inthe absence of leucocytes, a positive culture is unlikely and it also representsinadequate sampling(15).

Culture:

Qualitative culture:

There is high possibility of false positivity in Qualitative endotracheal aspirateculture .This is because of the growth of lower respiratory tract colonizers.(31)It is used to rule out the VAP diagnosis if negative culture is obtained.(15)

The treatment based only on qualitative culture report will result in unnecessary overuse of antibiotics.(46,47)

Semiquantitative culture:

Semiquantitative culture is performed based on the four quadrantstreak technique using a calibrated loop. Endotracheal aspirate (ETA)cultures are read semiquantitativelybyobservingthe growthin thefourquadrants, which suggests the approximate number of CFU/ml of thebacteria in the specimen(46).In a study comparing the semiquantitative culture (calibrated looptechnique) and the quantitative culture (serial dilution technique) of121 BAL samples, a very good agreement between the techniques wasobserved with only one discordant result[52]However, use of semiquantitative cultures for guiding antibiotictherapy may be associated with substantially more patients beingovertreated as observed in a study by Brun-Buisson et al(46).

Quantitative culture:

Quantitative culture is done by serially diluting the specimen.Culturereportsare given innumber of colony forming units per milliliter (CFU/ml).If it is more than the threshold value,it is diagnosed as pneumonia. The commonly used threshold values for diagnosis of VAP by quantitative culture are$\geq 10^5$for ETA,$\geq 10^4$for BAL,and$\geq 10^3$CFU/ml for PSB, respectively(4,48)Quantitativecultures are preferredfor making decisions regarding treatment of VAP.

Bronchoscopic specimens:

TheBAL and protected specimen brush (PSB) are the commonly used bronchoscopictechniques .(4) In critically ill patients ,there is mild risk for development of hypoxemia, cardiac arrhythmias, and bronchospasm(4).

Non bronchoscopic specimens- Endotracheal aspirates:

Endotracheal aspirates(ETA) cultured quantitatively is a good method to diagnose VAP asit is an non invasive approach which is inexpensive and can be used widely.(15).

Non-bronchoscopic vs. bronchoscopic specimens:

Quantitatively cultured Endotracheal aspirate and bronchoscopically collected specimens have a very good correlation.

Role of blood and pleural effusion cultures:

Though the organisms recovered from blood and pleural fluidis considered significant in VAPpatients,it has limited role in diagnosis because in only less than 10% VAP patients, spread occurs to blood and pleural space.(4)

The blood culture has a sensitivity of only 26%fordiagnosing the VAPpathogens.(49).Hence it is recommend to take blood and pleural effusion culturesin suspected VAP patients,when unable to find the other source of infection(4).

Role of biomarkers as diagnostic and prognostic markers of VAP:

Biomarkers likeprocalcitonin (PCT),C-reactive protein(CRP),endotoxin, soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) are used as diagnosticbiomarkers whereas proadrenomedullin, endothelin-1 precursor peptides andcortisol levels are used as prognostic markers.(50).

Treatment:

The guidelines suggested by American Thoracic Society forVAP treatment depends on the presence or absence of risk factors for MDR pathogens which is summarized below.(2).

Initial Empirical Treatment for VAP(2)	
VAP without any risk factors for MDR pathogens	**VAP associated with risk factors for MDR pathogens**
Ceftriaxone Or Levofloxacin,Moxifloxacin or Ciprofloxacin Or Ampicillin/Sulbactum Or Ertapenem	AntipseudomonalCephalosphorin(Cefipime ,Ceftazidime) Or AntipseudomonalCarbapenem(Imipenem or Meropenem) Or Beta Lactum/Beta lactamase inhibitor(Piperacillin-Tazobactum) Plus Antipseudomonalfluoroquinolone

	(Ciprofloxacin or Levofloxacin) Or Aminoglycoside (Amikacin,gentamicinorTobramicin) Plus Linezolid or Vancomycin (In MRSA infection.)
MDR=Mutidrug resistant, MRSA=Methicillin-resistant *staphylococcus aureus*	

The durationof empiric antibiotic therapy is traditionally fourteen to twenty one days,maybe shortened in those patients with good clinical recovery to7 days, except in case of infection withnonfermenters especially*Pseudomonas aeruginosa and Acinetobacterspecies*.(3)

Initial Intravenous adult dose of antibiotics for empiric therapy of VAP with late onset disease or risk factors for MDR pathogens(2)

Antibiotic	**Dosage ***
AntipseudomonalCephalosphorin	
Cefipime	1-2 g every 8-12 h
Ceftazidime	2g every 8h
AntipseudomonalCarbapenam	
Imipenem	500mg every 6h or 1g every 8h
Meropenem	1g every 8h
Beta Lactum/Beta lactamase inhibitor	
Piperacillin-Tazobactum	4.5 g every 6h
Antipseudomonalfluoroquinolone	
Ciprofloxacin	400mg every 8h
Levofloxacin	750mg every day
Aminoglycoside	
Amikacin	20mg/kg per day

Gentamicin Tobramicin Linezolid Vancomycin	7mg/kg per day 7mg/kg per day 600mg every 12h 15mg/kg every 12h

MDR=Multidrug resistant.
*Dosages are based on normal renal and hepatic function.

A new approach based on‘de-escalation’strategyhasbeen suggested for effective treatment without the antibioticoveruse.(51)

The use of clinical and microbiological data to change from an initial broad spectrum treatment to therapy witha narrower spectrum agents and withfewer antibiotics is referred as (51).In a study evaluating the nebulized colistin treatment, it is found to be safe and effective for treating Multi drug resistant organisms like *Acinetobacterbaumannii and pseudomonas aeruginosa*.(52).

Prevention of VAP:

There are multiple recommended measures for prevention of VAP.These measures are summarized below(1). Institutions or ICUs may observe a reduction in VAP rates by utilizing a ‘VAP-bundle’ approach. (54,55,57,58).

Suggested measures for prevention of ventilator-associated pneumonia- (1,56)

S.no.	ICU focused measures	Institution focused measures
1	Alcohol-based hand washing policy .	Surveillance program for pathogen profiling and creation of “antibiogram”
2	Early discontinuation of invasive devices	Frequent educational programs to Reduce unnecessary antibiotic prescription.
3	Reduce reintubation rates	Propagate use of non-invasive positive pressure ventilation(NIPPV)
4	Use of oropharyngeal vs. nasopharyngeal feeding tubes	Endotracheal tubes (ETTs) with potential benefit Polyurethane-cuffed ETT

		Silver/antibiotic coated ETT Aspiration of subglottic secretions.
5	Semi-recumbent patient positioning (30–45°)	Maintain policy for oral decontamination, Selective digestive decontamination (SDD)
6	Endotracheal tube cuff pressure ~ 20 cm H2O	Early weaning and extubation
7	Small bowel feeding instead of gastric feeding	Daily sedation holds
8	Prophylactic probiotics	Preference on using heat-moisture exchangers over heater humidifiers
9	Early tracheostomy	Mechanical removal of the biofilm (e.g., the mucus shaver)

The 5-element (Institute of Healthcare Improvement)IHI VAP bundle (57).includes:

1.oral care with chlorhexidine

2.Head of bed elevation,

3.stress ulcer prophylaxis,

4.daily sedation assessment and spontaneous breathing trials.

5.deep venous thrombosis prophylaxis.

Implementation of VAP prevention bundle significantly reduce VAP rates, antibiotic use and MRSA acquisition (53).

The IHI emphasizes the need for high (95 %) overall compliance rates with VAP bundles although this particular study reported overall bundle compliance rates of 70%.

A single-dose of antibiotics within four hrs of intubation may be effectivein a cohort of comatose patients in preventing early onset VAP (59).

MATERIALS AND METHODS:

Ethical consideration:

This study was approvedbyinstitututionalethicscommittee and informed consent was obtained from the study population.

Study design: Cross sectional study.

Study period: The study period is from October2014 to August 2015

Study setting:

The study was conducted at the Institute of Microbiology, Madras Medical College in association with other Departments (Intensive Medical Care Unit (IMCU)&Toxicology Unit) of Rajiv Gandhi Government General Hospital, Chennai.

Sample size: 100 patients

Study population:

The study was done in patients on ventilatory support for more than 48hrs in the IMCU & Toxicology Unit with the following inclusion criteria.

Inclusion criteria:

- Patients older than 18 years.
- Patients undergoing mechanical ventilation for more than 48hrs,with the radiological and clinical parameters indicative of Ventilator Associated pneumonia. (The parameters are presence of a new or progressive radiographic infiltrate plus atleast two of the following features which include fever greater than 38℃,leucocytosis or leukopenia and purulentlower respiratory secretions)(2)

Exclusion criteria:

- Patients who are severely immunocompromised such as Acquired immune deficiency syndrome(AIDS), organ transplant patients, terminal stages of malignancy are excluded.
- Patients with pneumonia prior to mechanical ventilation or within 48 hours of Mechanical ventilation.(2)

Data collection

The various patient data such as age, gender,address, date of admission ,level of consciousness,risk factors(presence of nasogastric tube,enteralnutrition,antacid or histamine type 2(H2) blocker therapy) involved, underlying diseases, date of intubation/ tracheostomy, duration of mechanical ventilation, prior antibiotic therapy etc.wererecorded.The clinical condition ofpatientswasfollowed up from the time of inclusion in the study to the date of discharge from MICU& Toxicology unit.

Sample Collection,Transport and processing:

Samples collected:

1.Endotracheal Aspirate

2.Bronchioalveolar lavage.

3.Blood.

Under strict aseptic precautions,samples were collected from the patients and transported immediately to the laboratory in appropriate settings and sample processing done.

Collection of Endotracheal aspirates(ETA):

Under aseptic precautions endotracheal aspirates were obtainedusing a 22-inch,No.12F suction catheter and collected in a mucus collector. The catheter was gently introduced through the endotracheal tube for at least 25-26cmlength.Gentle aspiration was then performed without instilling saline and the catheter was withdrawn from the ET tube, 2mL of normal saline was injected with a sterile syringe to flush the exudate into a sterile container for collection.(16)

Collection of Broncheoalveolarlavage(BAL):

During this procedure,a high volume of saline (100 to 300ml) was infused in to a lung segment through a bronchoscope by bronchoscopist,to obtain cells and proteins of pulmonary interstitium and alveolar spaces.It is estimated that more than one million alveoli are sampled during this process.The saline is then aspirated in a sterile containerand sent for microbiological processing.(60)

Processing of samples:

Respiratory(ETA&BAL) Samples were mechanically homogenised by vortexing for 1 min and then subjected to the following microscopic examination using standard laboratory techniques.(62)

Microscopy :

Direct Gram stain:

Direct examination of Gram stained preparations were performed and studied for the presence of squamous cells, polymorphonuclearcells,bacteria(Gram positive and Gram negative) and their morphology.

For Gram stain results, the thresholds for the diagnosis of VAP with the ETA samples were as follows:(5)

- >10 polymorphonuclear neutrophils (PMN) / high power field (HPF)
- ≥ 1 bacteria / oil immersion field .
- presence of intracellular bacterial inclusions.

Criteria used to reject endotracheal aspirates from adult patients by Gram's stain:(60)

1.Greater than 10 squamous epithelial cells per low power field.

2.No organism seen under oil immersion field.

KOH mount:

10% potassium hydroxide (KOH) mount is performed for the identification of fungal pathogens.(60)

Culture:

Endotracheal aspirate (EA) /BAL specimenswere processed quantitatively for the identification and categorisation of pathogens and colonizers.Specimenswereserially

diluted with sterile normal saline as 1/10dilution, 1/100dilution, 1/1,000 dilutions and 0.01 ml of above dilutions were inoculated on to 5% sheep blood agar,Macconkey agar and Chocolate agar. After incubation at 37℃ for 24 to 48 hours,colony count was done and expressed as number of colony forming units per ml (CFU/ml).(18).

The number of bacteria in the original sample is expressed in colony forming unit per millilitre.(cfu/ml)=number of colonies ×dilution factor×Inoculation factor.

Bacterial growth with a colony count $\geq 10^5$ CFU/ ml (for Endotracheal aspirate) and $\geq 10^4$cfu/ml(for BAL) were considered aspathogens.Growth of any organisms below the threshold were categorised as colonizers or contaminants.(4,16).

The plates whichshowed threshold growth were studied by colony morphology, Gram reaction and identified using standard biochemical reactions.Afterinitial characterisation of the isolates by colony morphology and Gram stain, species identification and susceptibility testing were done.

The sample was also inoculated on to two tubes of Sabouraud's dextrose agar and incubated at 25°C and 37°C.The slants were inspected daily during the first week and twice weekly during the next three weeks for growth.

The macroscopic appearance of the colonies in SDA were studied and the yeast isolates wereidentified by Gram's stain morphology andgerm tube test.

Blood culture:(62).

Under strict aseptic precautions, venepuncture site was cleaned with 70% alcohol and then with 2 % Povidone Iodine. The disinfectant was allowed to act for 1

minute and then 10ml of blood sample was collected with a sterile syringe and added into a sterile screw capped blood culture bottle containing 50 ml of sterile Brain Heart Infusion broth(BHI broth) at the bed side and transported immediately to the laboratory.

Brain Heart Infusion (BHI) Broth was incubated at 37°C aerobically and examined for turbidity at 24 and 48 hours. If turbidity or haemolysis was observed in BHI, subcultures were done onto Blood Agar and MacConkey Agar.

These plates were incubated aerobically at 37°C for 24 hrs. Any growth observed was identified up to species level by colony morphology, Gram staining, catalase test, oxidase test, motility and biochemical reactions.Subcultures were done every third day for a period of 10 days and a negative report was given if no growth was observed.

Interpretation of clinical and microbiological Criteria:

The patients satisfying both the clinical and microbiological criteria were diagnosed with VAP.(18,22)Modified clinical Pulmonary infection Score >6

Positive Gram stain (more than 10 polymorphonuclear cells/high power field and ≥ 1 bacteria per oil immersion field) and quantitative endotracheal aspirate culture results showing $\geq 10^5$CFU/ml.

VAP pathogenswere identified as follows:

Identification of the organisms were done by various biochemical tests like Catalase test, Oxidase test, Coagulase test, Nitrate reduction test, Indole test, Methyl red test,VogesProskauer test, Citrate utilization test, Urease test,Triple sugar iron agartest,Mannitol motility test and by standard bacteriological procedures.(62)

Gramnegative bacilli producing bluegreenpigment,Nonfermenting, motile, oxidase positive, nitrate reducing, were identifiedas*Pseudomonasaeruginosa.*

Gramnegativecoccobacilli ,Nonfermenting, non motile, oxidase negative, nitrate non-reducing, producing acid from OF glucose and 10% OF lactoseoxidatively, growth at 42° C, were identified as *Acinetobacterbaumannii.*

Gramnegative bacilli, fermenting glucose and othercarbohydrates,Oxidasenegative, catalase positive, nitrate reducing, nonspore forming, were identified as members of Enterobacteriaceae.

Gram-positive cocci in clusters, with characteristic golden yellow pigment,Catalasepositive, mannitol fermenting, coagulase producing were identified as *Staphylococcus aureus.*

Anti microbial susceptibility testing:

Anti microbial susceptibility testing is done by Kirby Bauer's disc diffusion method 0n Mueller Hinton agar based on CLSI guidelines.(62,63)

Preparation of inoculum for sensitivity testing:

A single colony of the test organism was picked up with sterile loop and suspended in saline and incubated at 37° C for 2 hrs.The turbidity of the suspension was adjusted to 0.5 McFarland's standard($1.5x10^8$ CFU/ml).The liquid culture of the test isolate adjusted to 0.5 McFarland turbidity was spread on the surface of Mueller Hinton agar plate.The plates were incubated at 37℃ overnight.The zone of inhibition was measured and interpreted as per CLSI (Clinical Laboratory Standards Institute) guidelines.The antibiotic discs were procured from Himedia,Mumbai and quality check done with the following control strains-

Escherichia coli(ATCC 25922),*Pseudomonas aeruginosa*(ATCC 27853)and*Staphylococcusaureus* (ATCC 25923)

Panel of antibiotics included for testing antimicrobial sensitivity of Gram negative bacilli.(63)

Antibiotic (Disc content)	**Diameter of Zone of inhibition in mm.**		
	Sensitive	**Intermediate**	**Resistant**
Amikacin (30 µg)	≥ 17	15-16	≤ 14
Cefotaxime (30 µg)			
Enterobacteriaceae	≥26	23-25	≤22
Acinetobacter sp.	≥23	15-22	≤14
Ceftazidime (30 µg)			
Enterobacteriaceae	≥21	18-20	≤17
P.aeruginosa&Acinetobacter sp.	≥18	15-17	≤14
Cotrimoxazole (1.25 µg / 23.75 µg)	≥16	11-15	≤10
Ciprofloxacin (5 µg)	≥21	18-20	≤17
Gentamicin (10 µg)	≥15	13-14	≤12
Imipenem (10 µg)			
Enterobacteriaceae	≥23	20-22	≤19
P.aeruginosa	≥19	16-18	≤15
Acinetobacter sp.	≥16	14-15	≤13

Meropenem(10µg)	≥ 18	15-17	≤14
Piperacillin- Tazobactam (100 µg/10 µg)	≥21	18-20	≤17

The panel of antibiotics included in the antimicrobial sensitivity testing for *Staphylococcus aureus*were .(63)

Antibiotics	**Disc content**	**Zone of inhibition in mm**		
		Sensitive	**Intermediate**	**Resistance**
Amikacin	30µg	≥17	15-16	≤14
Gentamicin	10µg	≥15	13-14	≤12
Ciprofloxacin	5µg	≥21	16-20	≤15
Cotrimoxazole	1.25/23.75µg	≥16	11-15	≤10
Chloramphenicol	30µg	≥18	13-17	≤12
Penicillin	10units	≥29	-	≤28
Erythromycin	15µg	≥23	14-22	≤13
Tetracyclin	30µg	≥19	15-18	≤14
Cefoxitin	30µg	≥22	-	≤21

The VAP pathogens were screened for the production of (ESBL) Extended spectrum beta lactamases.

Extended spectrum ß- lactamase (ESBL) detection method:

Gram negative bacilli (Enterobacteriaceae family) showing reduced zone of inhibition around Ceftazidime(30µg) &Cefotaxime(30µg) discs were further confirmed by combination disc method.

Antibiotic	**Zone diameter for ESBL producing strain**
Ceftazidime(30µg)	≤22mm
Cefotaxime(30µg)	≤27mm

Phenotypic confirmation method:

Combination disc test:

Procedure:

Using a sterile loop, four or five colonies of similar morphology were picked up, inoculated to peptone water & incubated at 37° C for 2-4 hours until turbidity matched that of McFarland 0.5 turbidity standard (1.5 x 10^8cfu/ml) . Lawn culture was done on Mueller-Hinton agar plates and antibiotic disc ceftazidime(CAZ 30µg) and ceftazidime /clavulanic acid (CAZ/CA 30µg/10µg) discs were placed on to the plate.

Interpretation :

Zone of inhibition was measured around the disc.Anincrease of≥5mm in zone of inhibition in a disc containing clavulanic acid compared to the drug alone is considered as ESBL producer.(63)

AmpC beta lactamase enzyme detection:(64,65)

Screening method:

A 0.5 Mcfarland of the test isolate was swabbed on MHA plate and disc of cefotaxime(30µg),Ceftazidime(30µg) were placed adjacent tocefoxitin(30µg) at a

distance of 20mm from each other.Afterincubation,isolatesshowing blunting of ceftazidime or cefotaxime zone of inhibition adjacent to cefoxitin disc or showing reduced susceptibility to either of the above drugs andcefoxitin(30µg) were considered as "screen positive" and selected for detection of AmpC β-lactamases by AmpCdisc test.

AmpC Disc Test:

A lawn culture of E. coli ATCC 25922 was prepared on MHA plate. Sterile discs (6mm) were moistened with sterile saline (20µl) and inoculated with several colonies of test organism. The inoculated disc was then placed beside a cefoxitin disc (almost touching) on the inoculated plate. The plates were incubated overnight at 37°C. A positive test appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc. A negative test had an undistorted zone.

Metalloß lactamase (MBL) detection method:

Screening for MBL:

Isolates resistant tocarbapenem(Imipenem or Meropenem)were further subjected to confirmatory tests for MBL detection.

Imipenem-EDTA combined disc test:(66)

Two to three identical colonies of the test organism were inoculated into salineand incubated at 37° C for 4 to 6 hours to obtain optical density matching that of 0.5McFarland turbidity standards.

This suspension of test organism was then inoculated on to Mueller-Hinton Agar (MHA) plates by performing lawn culture with a sterile cotton swab.Imipenem (10µg) disc and (10 µg)Imipenem disc containing 750 µg of EDTA were placed 20mm apart in the plate.

Afterovernight incubation at 37°C,enhancement of the zone of inhibition of Imp-EDTA combination disc of ≥7mm when compared to Imipenem disc alone was interpreted as a positive result(MBL production).

Modified Hodge Test:(63)

Two to three identical colonies of Escherichia coli (ATCC 25922) were inoculated into saline and incubated at 37° C for 4 to 6 hours to obtain optical density matching that of 0.5 McFarland turbidity standards.

A lawn culture of E.coli ATCC 25922 was done on to the Mueller-Hinton Agar (MHA) plates with a sterile cotton swab. A 10µg Meropenem disc was placed at the centre and the test organism was streaked in a straight line from the edge of the disc to the edge of the plate. The plate was incubated overnight at 37° C.

The presence of distorted zone of inhibition or clover leaf type of indentation at the intersection of the test organism and E.coli 25922, within the zone of inhibition of the Meropenem susceptibility disc was interpreted as positive result.

METHODS FOR DETECTION OF MRSA:

Cefoxitin disc method:(63)

0.5 Mcfarland's suspension of test isolate and Staphylococcus aureus ATCC 25923 was lawn cultured on cation adjusted MHA plates separately. 30µgcefoxitindisc was

placed on the surface of lawn culture of both isolates and incubated at 33–35 °C in ambient air for 16–18 hours.

The Interpretation was done as follows:

For *Staphylococcus aureus:*

Zone of inhibition :≥22mm-MSSA (*mec A* negative)

Zone of inhibition :≤21mm-MRSA(*mec A* positive)

MINIMUM INHIBITORY CONCENTRATION BY EPSILOMETER TEST (E-TEST): (62)

All MRSA isolates were subjected to MIC estimation for Vancomycin, by using E-test(Epsilometer) method (HI-MEDIA).

The E-test strips contains antimicrobial agent with a continuous exponential gradient of antibiotics immobilized on paper material and MIC values printed on both sides identically.

Procedure:

The strains were inoculated into tubes containing 2ml of peptone water. The suspension was streaked onto the Mueller Hinton Agar with 2% Nacl to give a lawn culture. E-test strips were placed on the inoculated plates.The plates were incubated at 37°C for 24 hours and reading was taken the next day.

MIC of the drug was taken at the point where the ellipse intersects the MIC scale on the strip. Control strain ATCC Staphylococcus aureus25923 were tested in parallel.

MINIMUM INHIBITORY CONCENTRATION BY MACROBROTH DILUTION METHOD:(for vancomycin and meropenem)

Preparation of stock antibiotic solution: (62)

Antibiotic stock solution was prepared using the formula

$$\frac{1000}{P} \times V \times C = W.$$

Where P= potency of the antibiotic in relation to the base.

(For vancomycin, P= 950/1000 mg; Himedia)

(For Meropenem, P=750/1000mg)

V = volume of the stock solution to be prepared (10ml)

C =final concentration of the antibiotic solution (1024µg/ml)

W = weight of the antibiotic to be dissolved in the volume V.

In a sterile screw capped bottle,10ml of distilled water is taken and the appropriate weight of drug (eg.vancomycin,Meropenem) is added to prepare stock solution with a final concentration the of antibiotic solution as 1024 µg/ml.

Inoculum preparation for the test and ATCC control strain:

To 9.9 ml of Mueller Hinton broth in a sterile container , 0.1 ml of 0.5 Mcfarland turbidity matched test organism was added and mixed well.Similarly ATCC control strain inoculum was prepared.

Procedure:

Two rows of 13 sterile plugged test tubes were arranged in the racks. Using a fresh pipette, 1ml of peptone water was added to all the tubes starting from 1st to 13thtube.The contents of the container with stock solution were mixed thoroughly and using a sterile pipete,1ml of the stock solution was transferred to first tube in

each row,mixedwell and from this concentration(512µg/ml), 1ml was transferred to the second tube (256µg/ml) ,then it is serially diluted till the last row.The various concentration of antibiotics in the following tubes are 128,64,32,16, 8, 4, 2, 1, 0.5, 0.25, 0.125(µg/ml).Using sterile pipette, 1 ml of the above inoculum was transferred to each antibiotic containing tubes in the first row and also to the growth control tube.

The first row of tubes were inoculated with test organism.

The secondrow of tubes were inoculated with ATCC control strain.

ATCC *Pseudomonas aeruginosa* 27853 was used as the control strain for testing Meropenem.ATCC*Staphylococcus aureus*25923 was used as the control strain for testing Vancomycin.

1 ml of the antibiotic free broth was placed in the last tube in each row as growth control.

1 ml of antibiotic solution were kept as sterility control.

These tubes were incubated at 37°C overnight.

Observation &Interpretation:

The MIC of ATCC control strain were observed, they were within sensitive range, hence the test was considered to be valid. The lowest concentration of the antibiotic in which there was no visible growth was taken as the MIC of the drug for the test organism.

Interpretation:Minimum Inhibitory Concentration(MIC).

Drug	**Susceptible**	**Intermediate**	**Resistant.**
Vancomycin	≤ 2µg/ml	4-8µg/ml	≥16µg/ml

Meropenem	≤ 2μg/ml	4μg/ml	≥ 8 μg/ml

Statistical analysis:

SPSS for windows Version SPSS 20 is used for data entry analysis. All P values <0.05 were considered to be significant statistically.

RESULTS:

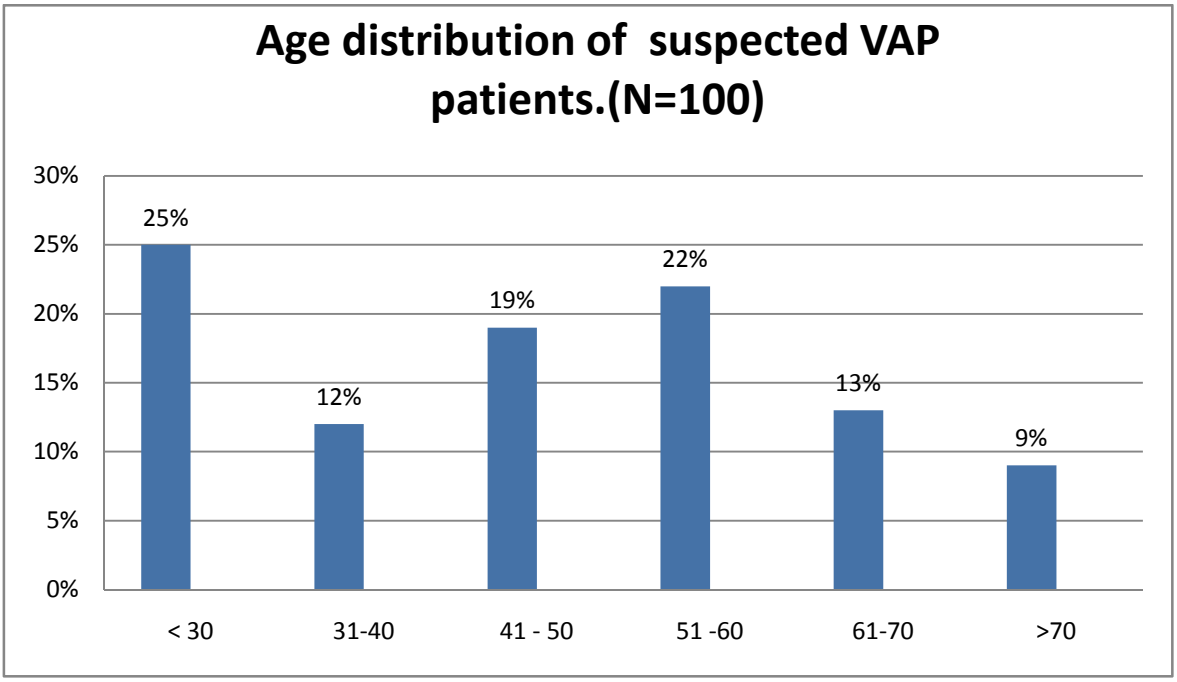
This study was conducted in the MICU setting of Government General Hospital. Chennai from October 2014 to August 2015.

A total of 100 patients who full filled the inclusion criteria were taken into the study.

Table1:Ageand Gender distribution of suspected VAP patients.(n=100).

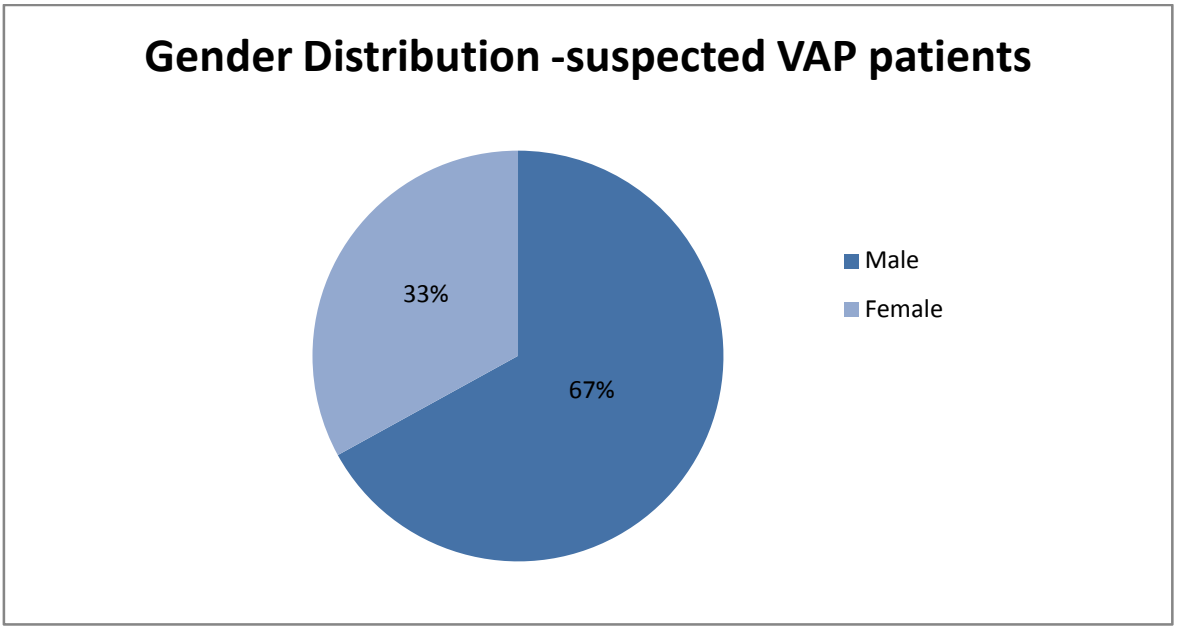
Age Group	Male	Female	Total	Percentage
< 30	13	12	25	25%
31-40	7	5	12	12%
41 – 50	12	7	19	19%
51 -60	18	4	22	22%

61-70	9	4	13	13%
>70	8	1	9	9%
Total	**67**	**33**	**100**	**100%**



Among the suspected VAP patients,majority(25%) of patients belong to less than 30 years and 22% belongs to 51-60 years.

Gender Distribution of suspected VAP Patients.(N=100)



Out of the 100 patients included in the study,67 (67%) were males &33(33%) were females.

Table-2 Clinical Spectrum of Patients included in the study(N=100)

Diagnosis	Total N=100	Percentage
Poisoning	33	33%
Cardiovascular Diseases	17	17%
Intra-abdominal diseases	13	13%
Neurological Disorders	11	11%
Sepsis	10	10%
CNS Infections	9	9%
Head injury	5	5%
Neurotoxic Snakebite	2	2%

The clinical spectrum of patients included in the study was shown in Table-2.It indicates that the maximum number of cases enrolled in the study were of poisoning (33cases) followed by Cardiovascular diseases (17),Intra abdominal diseases(13),Neurological disorders(11),Sepsis (10),CNS infections (9),head injury(5),Neurotoxic snake bite(2)

Clinical Spectrum of Patients included in the study:(N=100)

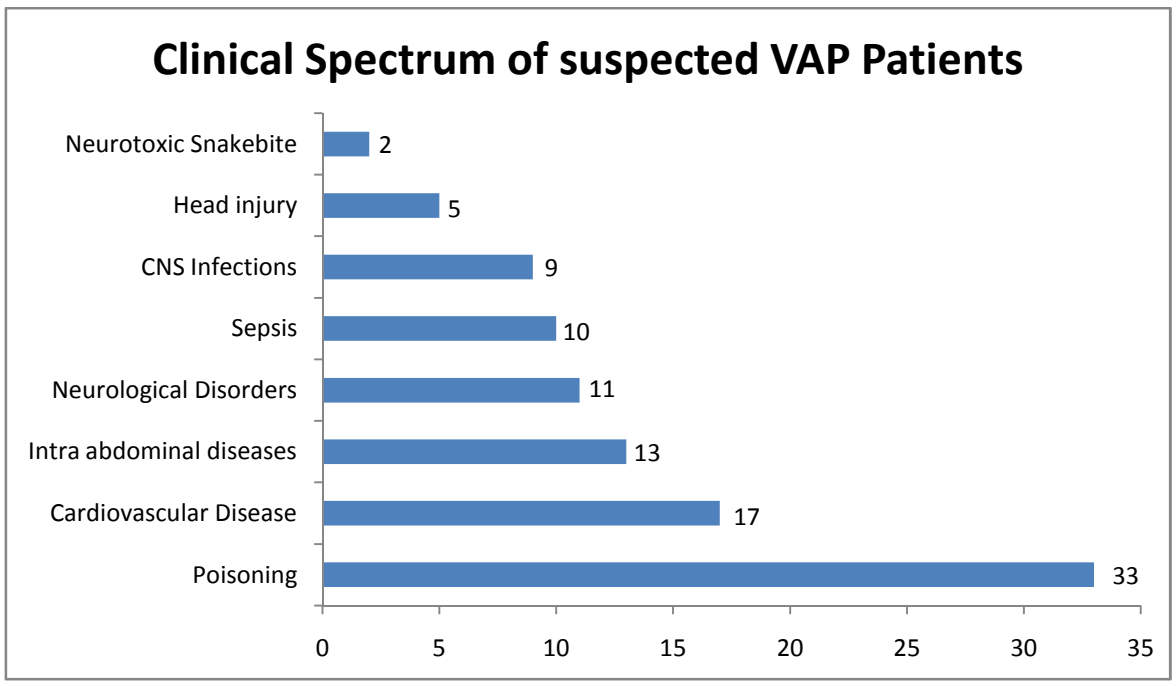


Table 3 Distribution of samples among the patients(N=100)

S.no	Samples	Count

1	Respiratory Sample a. Endotracheal aspirates(ETA)	100
	b. BAL(Broncheoalveolar lavage)	11
2	Blood	100

Both endotracheal aspirates and Bronchioalveolar lavage specimen were collected from 11 patients out of 100 patients.

Table 4 Correlation between pus cells in Gram stain and growth in quantitative culture:

Gram Stain		Quantitative Culture of ETA					Total
		≥ 10^5 Cfu/ml (Pathogens) N=23		<10^5Cfu/ml (colonizers) N=44		NG N=33	
No. of pus cells/HPF	>10	19	83%	6	14%	0	29
	1-10	4	17%	31	70%	9	40
	0	-	-	7	16%	24	31

Respiratory Samples.	**Quantitative culture.**

	Threshold of Pathogens		Threshold of Colonizers	
	ETA $\geq 10^5$ Cfu/ml	BAL $\geq 10^4$ Cfu/ml	ETA $<10^5$ Cfu/ml	BAL $<10^4$ Cfu/ml
Endotracheal aspirates (ETA).N=100	23	NA	37	NA
Broncheoalveolarlavage(BAL).N=11	NA	3	NA	7
Total	23		44	

Table 5 Results of Quantitative culture of respiratory samples.

Same pathogens were isolated from both endotracheal aspirates and bronchioalveolar lavage in 3 patients.

Correlation of Gram stain findings with quantitative culture.

Gram stain Findings	**Quantitative culture**			
	Pathogens	**Colonizers**		**NG**
Pus cells +,organism+	19(83%)	2	4.5%	0
Pus cells +,organism -	4(17%)	39	88.5%	5(15%)
Pus cells - ,organism +	0	3	7%	0
Pus cells -,organism -	0	0	0	28(85%)

The presence of >10 pus cells /HPF with ≥1 bacteria per oil immersion field is an useful method for presumptive diagnosis of VAP.

Table7 Clinical Pulmonary Infection Score (CPIS Score)

CPIS Score	No of Patients
≤6	77
>6	23

The patients with CPIS score of >6 were diagnosed as VAP patients.

The mean CPIS of confirmed VAP cases(8.48±1.238) were significantly higher than that of No VAP group (3.55±0.804)(The two tailed p value is <0.0001).

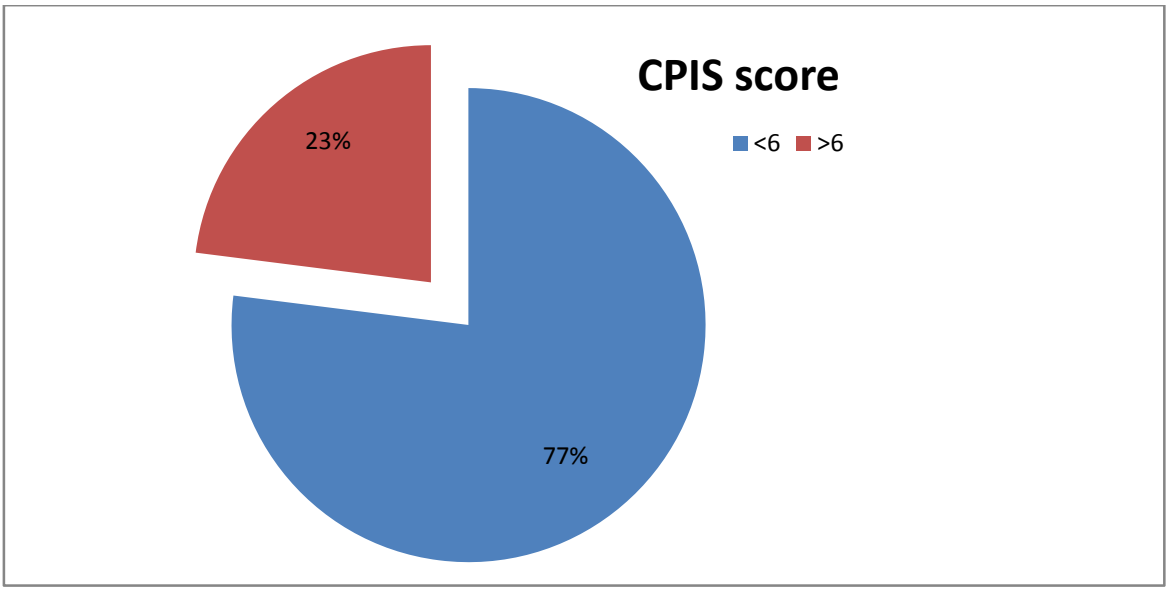


Table 8 Calculation of VAP rate per 1000 ventilator days:

Month	No of Patients on Mechanical Ventilation.	Duration of mechanical ventilation (In days)	No. of VAP cases diagnosed.	VAP Rate per 1000 ventilator days.

December 2014	118	263	4	15
January 2015	109	206	3	14.5
February 2015	91	212	4	18.8
March 2015	104	209	3	14.4
April 2015	120	301	5	16.6
May 2015	112	242	4	16.5
Total	654	1433	23	16(average)

In this study ,the VAP rate was 16 per 1000 ventilator days

Table9 Age and Gender distribution of confirmed VAP patients.(N=23)

Age Group	Male N=17	Female N=6	Total N=23	Percentage
< 30	1	3	4	17%
31-40	2	0	2	9%
41 – 50	2	2	4	17%
51 -60	7	0	7	31%
61-70	3	1	4	17%
>70	2	0	2	9%

The age and gender distribution of confirmed VAP cases were studied and it was found that,the incidence of VAP was highest in patients of age between 51-60 years . Males(74%) were commonly affected than females(26%).

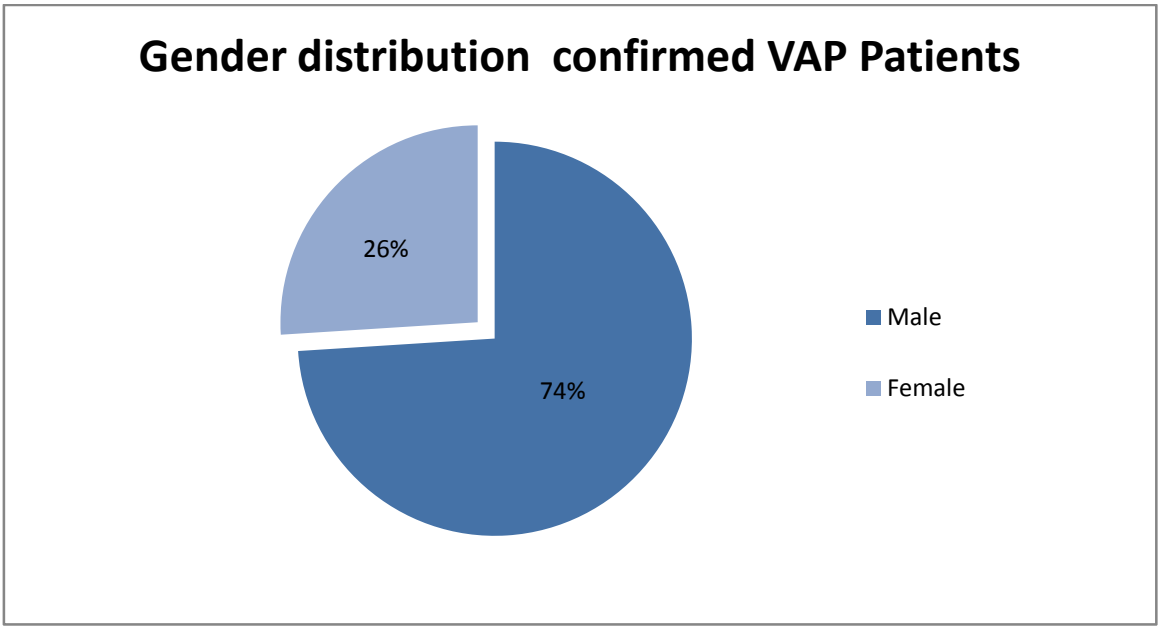


Table10 Clinical Spectrum of confirmed VAP patients.N=23

Diagnosis	**Total N=23**	**Percentage**
OPC Poisoning	7	30%
Cardiovascular Diseases	3	13%
Intra-abdominal diseases	4	17%
Neurological Disorders	2	9%
Sepsis	3	13%
CNS Infections	1	4%
Head injury	3	13%

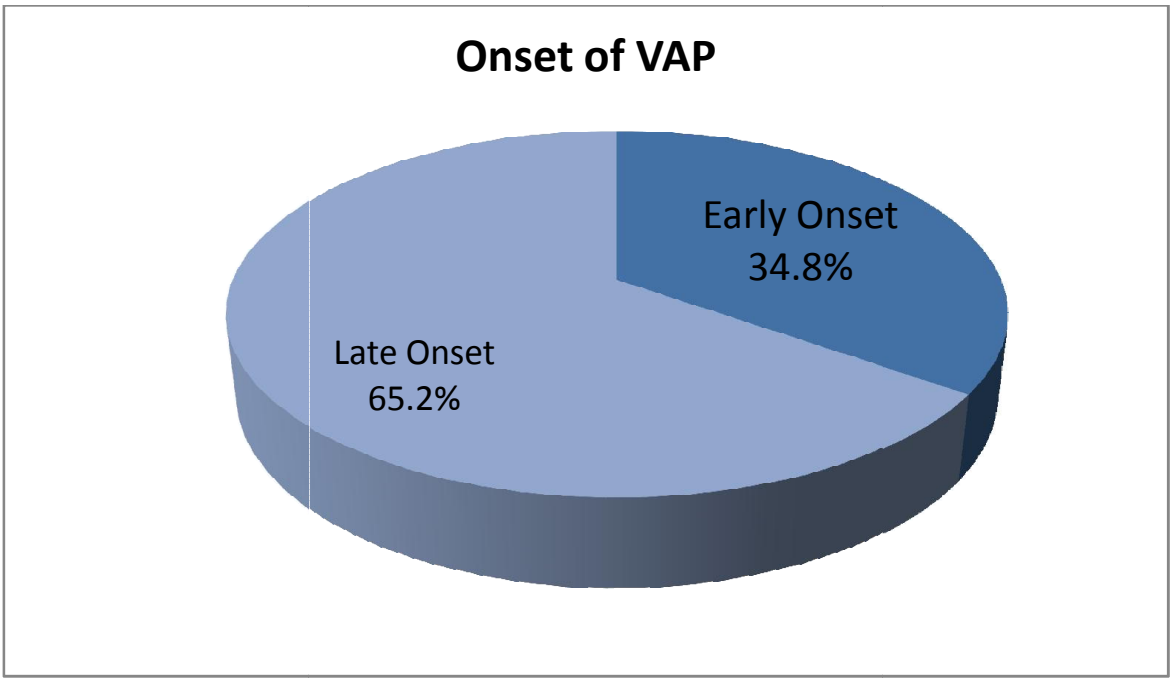
The highest percentage of VAP occurrence was seen among patients with Organo phosphorus poisoning (30%) followed by intra abdominal diseases (17%),Sepsis(13%),head injury(13%).

Table 11.VAP onset.

VAP Onset	Number of Patients (N-23)	Percentage	P value
Early	8	34.8%	0.144
Late	15	65.2%	NS

NS-Not Significant.

VAP onset.

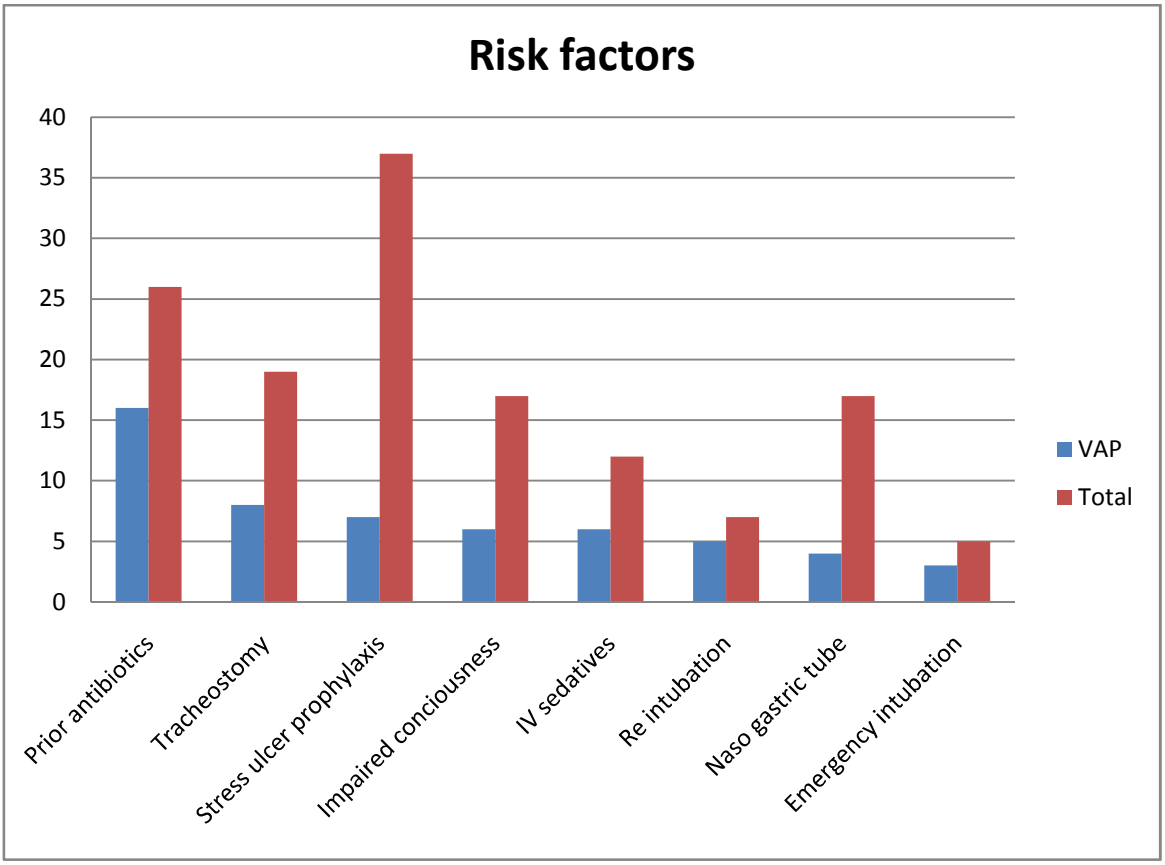


Out of 23 confirmed VAP patients, 8(35%) were categorised under early onset VAP & 15(65%) were categorised under late onset VAP.

Table 12 Risk factors in patients included in the study:((N=100)

Risk factors	Total	VAP	Percentage	Pvalue
Prior antibiotics	26	16	61.5%	0.023(S)
Tracheostomy	19	8	42%	NS
Stress ulcer prophylaxis	37	7	19%	NS
Impaired consciousness	17	6	35%	NS
IV sedation	12	6	50%	NS
Reintubation	7	5	71%	0.025(S)
Nasogastric tube	17	4	24%	NS
Emergency intubation	5	3	60%	0.045(S)

S-Significant,NS-Not significant.



VAP Pathogens:

The majority of the bacterial isolates were found to be gram negative bacilli(91%),of which Non fermentors(65%) were the predominant pathogens isolated from confirmed VAP patients in our study.The gram positive organism accounts for 9% of the VAP isolates ,of which all were methicillin resistant staphylococcus aureus. Of the 23 patients diagnosed as VAP pathogens,21 (91%) patients had monomicrobial infection and 2 (9%) patients had polymicrobial infection.

Table 13 VAP Pathogens(N=23)

Sr.no	VAP Pathogens(N=23)	Count. (N=23)	Percentage
1	Gram negative bacilli	21	91%
2	Gram positive cocci	2	9%

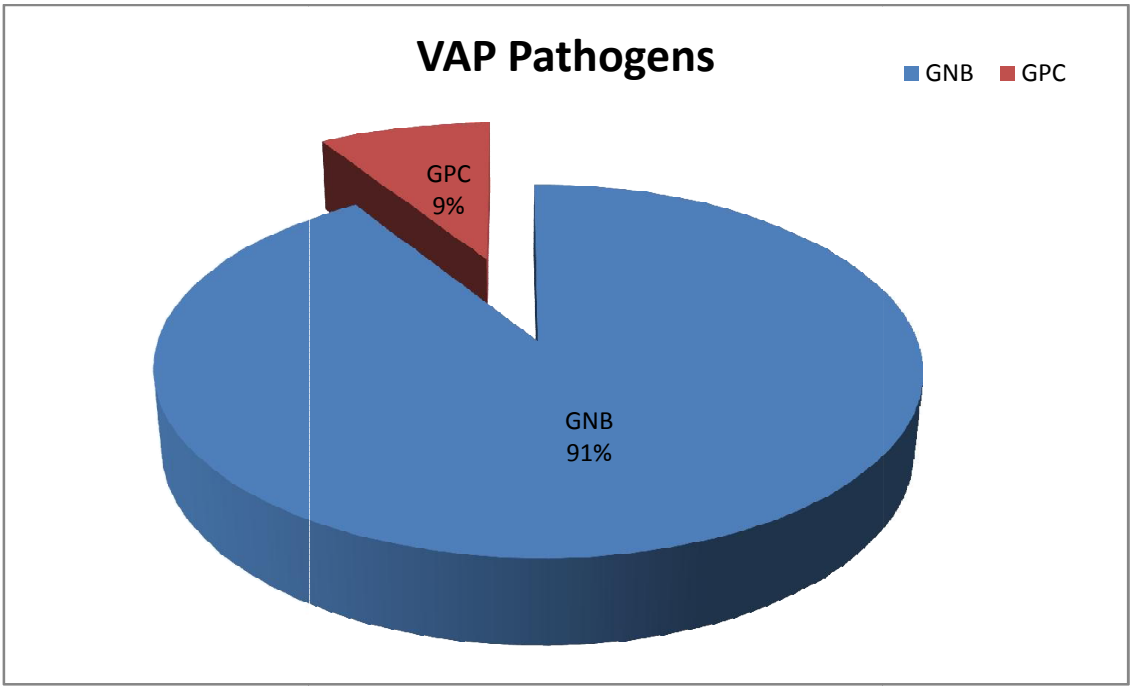


Table-14 - Etiological Agents of VAP(n=23)

Organism	**Total n=23**	**Percentage**
Acinetobacterbaumannii	9	39%
Pseudomonas aeruginosa	6	26%
KlebsiellaPneumoniae	3	13%
KlebsiellaOxytoca	2	9%

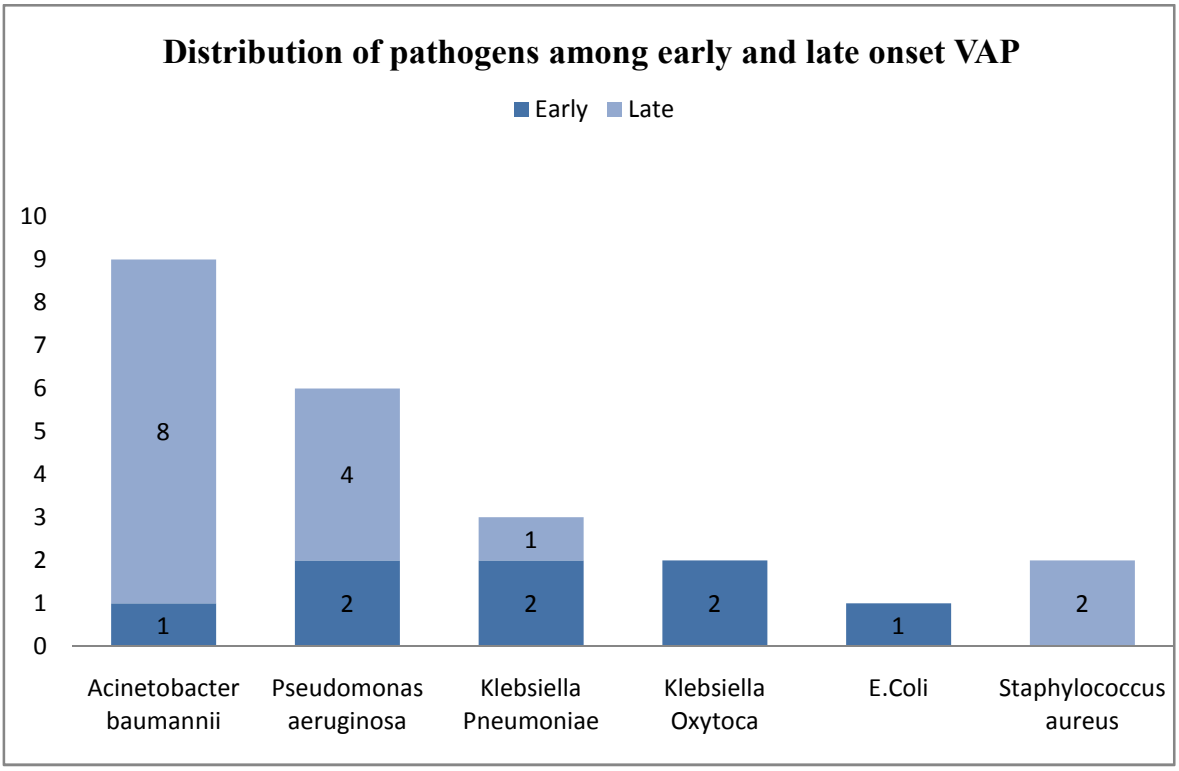
E.Coli	1	4%
Staphylococcus aureus	2	9%

The most frequently isolated organisms in VAP patients were *Acinetobacterbaumannii* (39%) followed by *Pseudomonas aeruginosa* (26%),*Klebsiellapneumoniae* (13%),*Klebsiellaoxytoca*(9%),*E.coli*(4%), *Staphylococcus aureus*(9%).

Table 15 – Distribution of pathogens among early and late onset VAP

Organism	**Early (n=8)**	**percentage**	**Late (n=15)**	**Percentage**
Acinetobacterbaumannii	1	12.5%	8	53%
Pseudomonas aeruginosa	2	25%	4	27%
KlebsiellaPneumoniae	2	25%	1	7%

KlebsiellaOxytoca	2	25%	0	-
E.Coli	1	12.5%	0	-
Staphylococcus aureus	0	-	2	13%



The predominant organism in the late onset VAP was *Acinetobacterbaumannii(*53%) followed by *Pseudomonas aeruginosa*(27%).The predominant organism in the early onset VAP group were *Pseudomonas aeruginosa*(25%),*Klebsiella pneumonia*(25%) and *klebsiellaoxytoca(*25%).

Table:16 Distribution of respiratory tract Colonizers in mechanically ventilated patients.(N=100)

Sno	Organism(Colonizer)	Count	Percentage
1	*Acinetobacterbaumannii*	10	23%
2	*Acinetobacterlwoffii*	3	7%
3	*KlebsiellaPneumoniae*	10	23%
4	*KlebsiellaOxytoca*	2	4.5%
5	*Pseudomonas aeruginosa*	8	18%
6	*Pseudomonas fluorescens*	1	2%
7	*Staphylococcus aureus*	4	9%
8	*Staphylococcus epidermidis*	2	4.5%
9	*Escherichia coli*	2	4.5%
10	*Candida albicans*	2	4.5%
	Total	44	23%

The common organisms colonizing the respiratory tract were *Acinetobacterspecies*(30%),klebsiella species(27.5%) and pseudomonas species (20%).*Staphylococcus aureus (*9%) was the common gram positive colonizer . *Staphylococcus epidermidis*, *Escherichia coli* and *Candida albicans* were the other relatively less common colonizers.

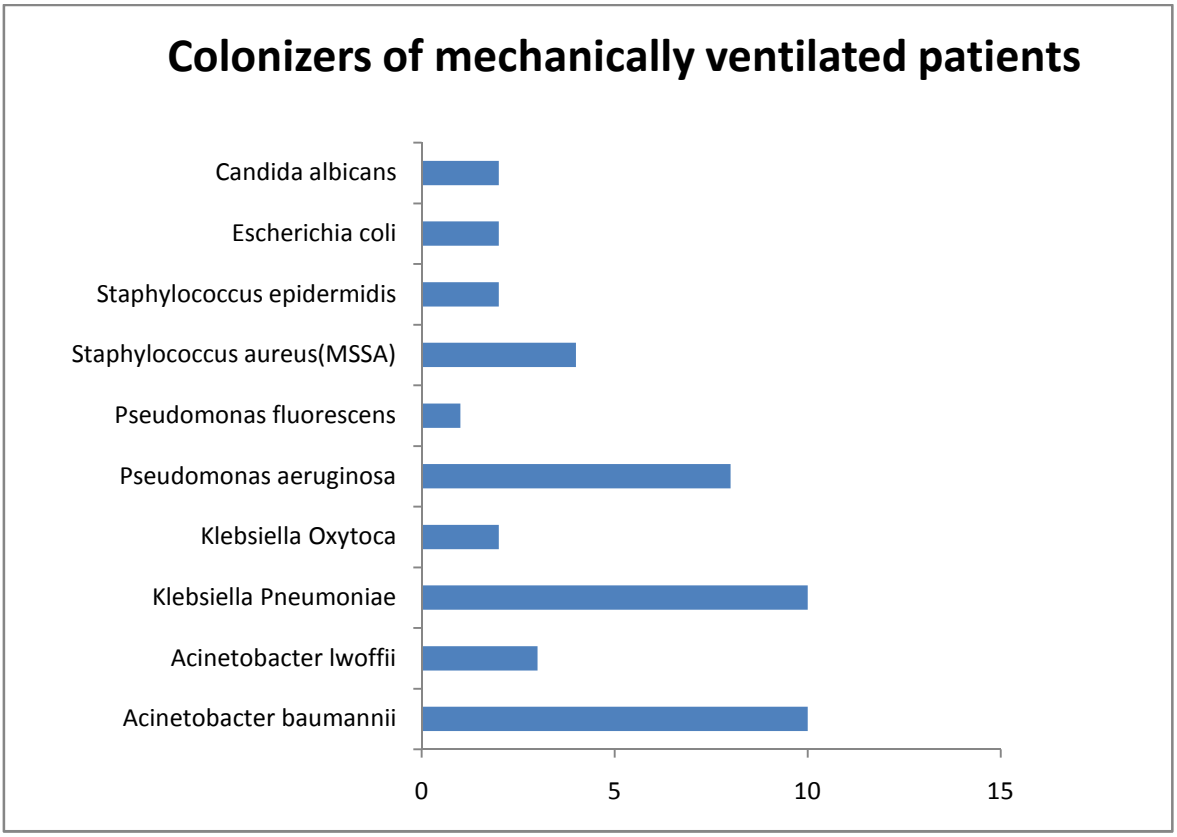


Table 17: Distribution of etiological agents causing bacteremiaamong confirmed VAP Patients (n=23)

S.no	Blood Culture	VAP	%	P value
1	*Acinetobacterbaumannii*	1	4.3	
2	*KlebsiellaPneumoniae*	1	4.3	
3	*KlebsiellaOxytoca*	1	4.3	0.721
4	*Pseudomonas aeruginosa*	2	9	Not

5	*Staphylococcus aureus*	3	13.1	Significant
	Total	8	35	

Blood culture:

Out of 23 VAP cases, blood culture was positive in 8 patients.The organisms isolated were *Acinetobacterbaumannii, KlebsiellaPneumoniae,KlebsiellaOxytoca, Pseudomonas aeruginosa, Staphylococcus aureus*.The sensitivity of blood cultures for the diagnosis of VAP is low and also if positive,the organisms may originate from an extrapulmonary site of infection.

Antimicrobial susceptibility pattern of the Gram negative and Gram positive isolates causing VAP is shown below.Most of the Gram negative organisms and gram positive organisms isolated were multidrug resistant.

Table 18: Antimicrobial sensitivity pattern of Gram negative isolates

Organism	**AK**	**COT**	**CIP**	**CTX**	**CAZ**	**CAC**	**CX**	**GM**	**IMP**	**MER**	**PT**
Acinetobacterbaumannii(n=9)	44% (4)	22% (2)	33% (3)	-	11%(1)	-	44%(4)	22% (2)	67% (6)	67% (6)	44% (4)

Pseudomonas aeruginosa (n=6)	67% (4)	-	17% (1)	-	33% (2)	-	50%(3)	50% (3)	61% (4)	61% (4)	50% (3)
Klebsiellapneumoniae (n=3)	67% (2)	0%	33% (1)	0%	0%	67% (2)	67% (2)	33% (1)	100% (3)	100% (3)	67% (2)
Klebsiellaoxytoca (n=2)	50% (1)	0%	50% (1)	0%	0%	100% (2)	100% (2)	50% (1)	100% (2)	100% (2)	100% (2)
E.Coli (n=1)	100% (1)	0%	0%	0%	0%	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)

AK-Amikacin,COT-Cotrimoxazole,CIP-ciprofloxacin,CTX-cefotaxime,CAZ-ceftazidime,CAC-Ceftazidime&clavulanic acid,CX-cefoxitin,
GM-Gentamicin,IMP-Imipenem,MER-Meropenem,
PT-Piperacillintazobactum.

The nonfermentors showing resistance to carbapenemswere further subjected to Macrobroth dilution method for determining the MIC.

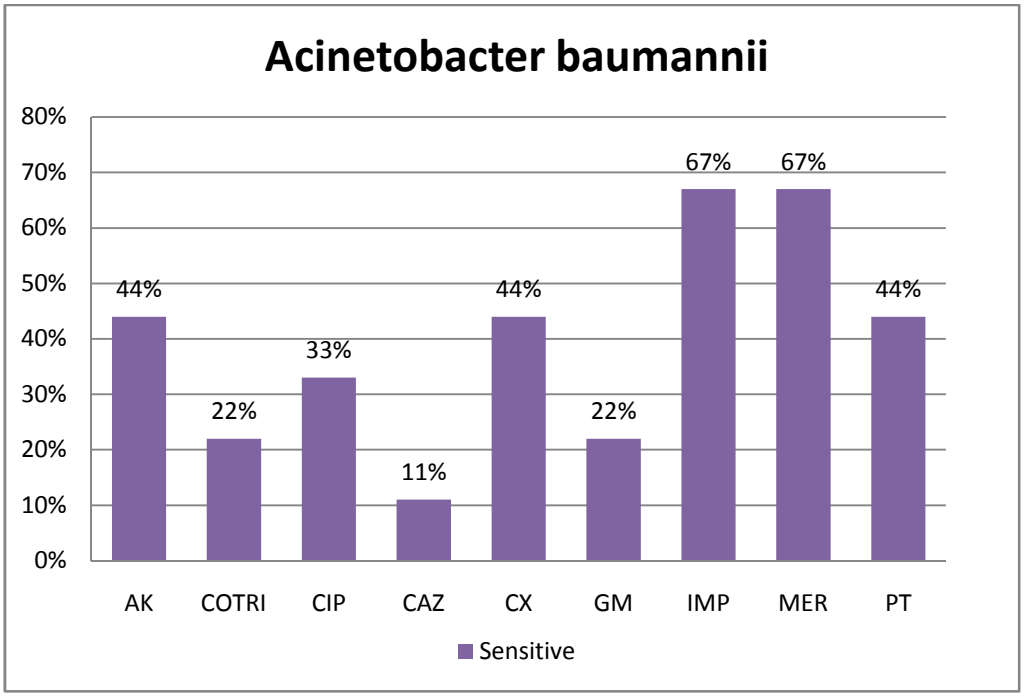
Table 19.Sensitivity to meropenem by Disc diffution method and Macrobroth dilution method

	Number of isolates resistant to Meropenem

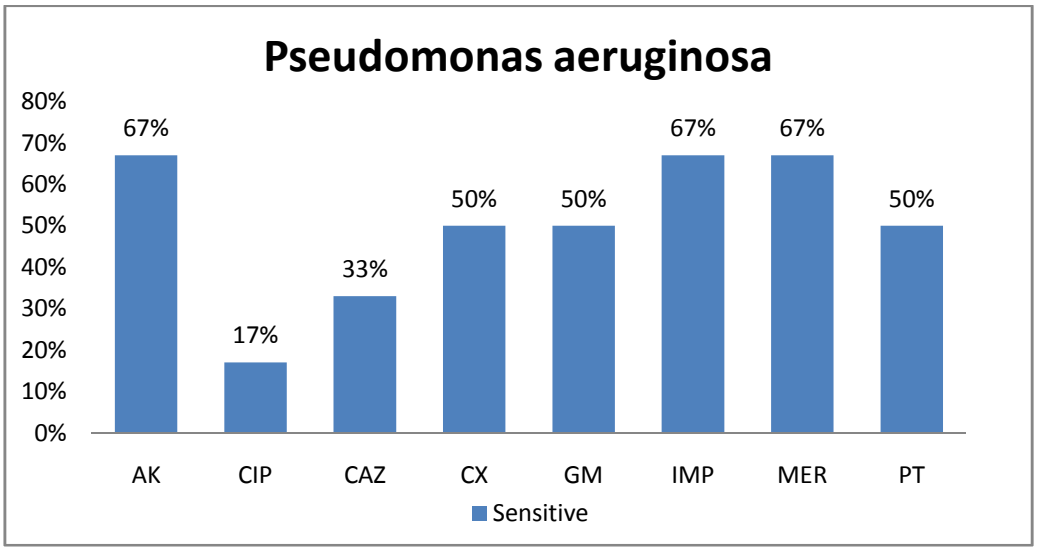
Method	*Acinetobacterbaumannii*.		*Pseudomonas aeruginosa*	
	Count	Percentage	Count	Percentage
Kirby Bauer's Disc Diffusion method.	3	33% (3/9)	2	33% (2/6)
Macrobroth dilution method	3	33%	2	33%

The isolates of nonfermentors showing resistance to carbapenem (meropenem) by Disc Diffusion method also showed resistance by Macrobroth dilution method with a MIC value of >8µg/ml.

Antimicrobial sensitivitypattern of *Acinetobacterbaumannii.*

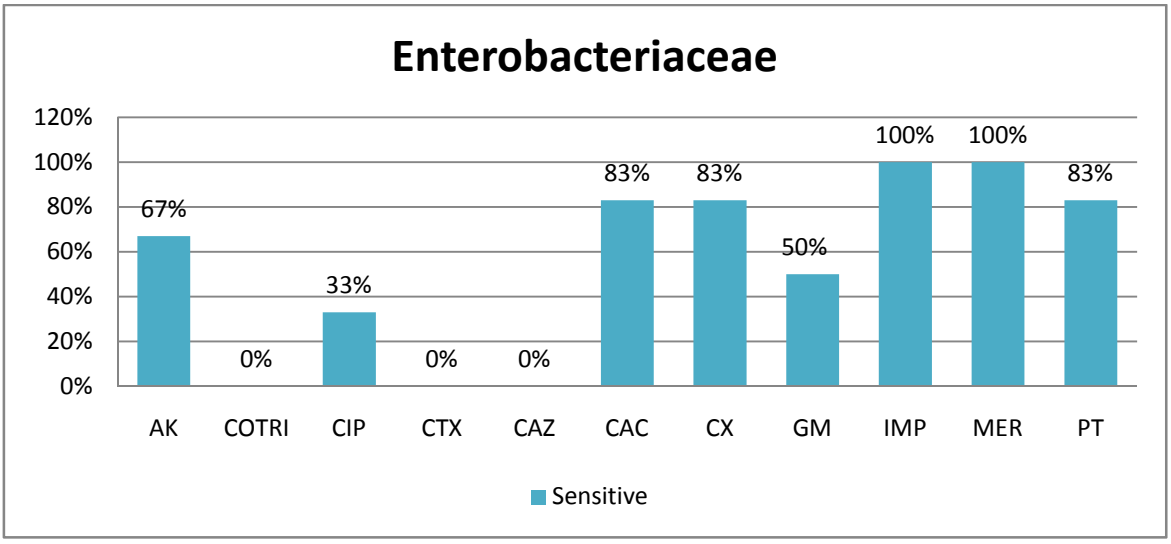


Antimicrobial sensitivitypattern of *Pseudomonas aeruginosa*



67% isolates of *Pseudomonas aeruginosa* were sensitive to amikacin,67% to carbapenems and 50% to Piperazilintazobactum.

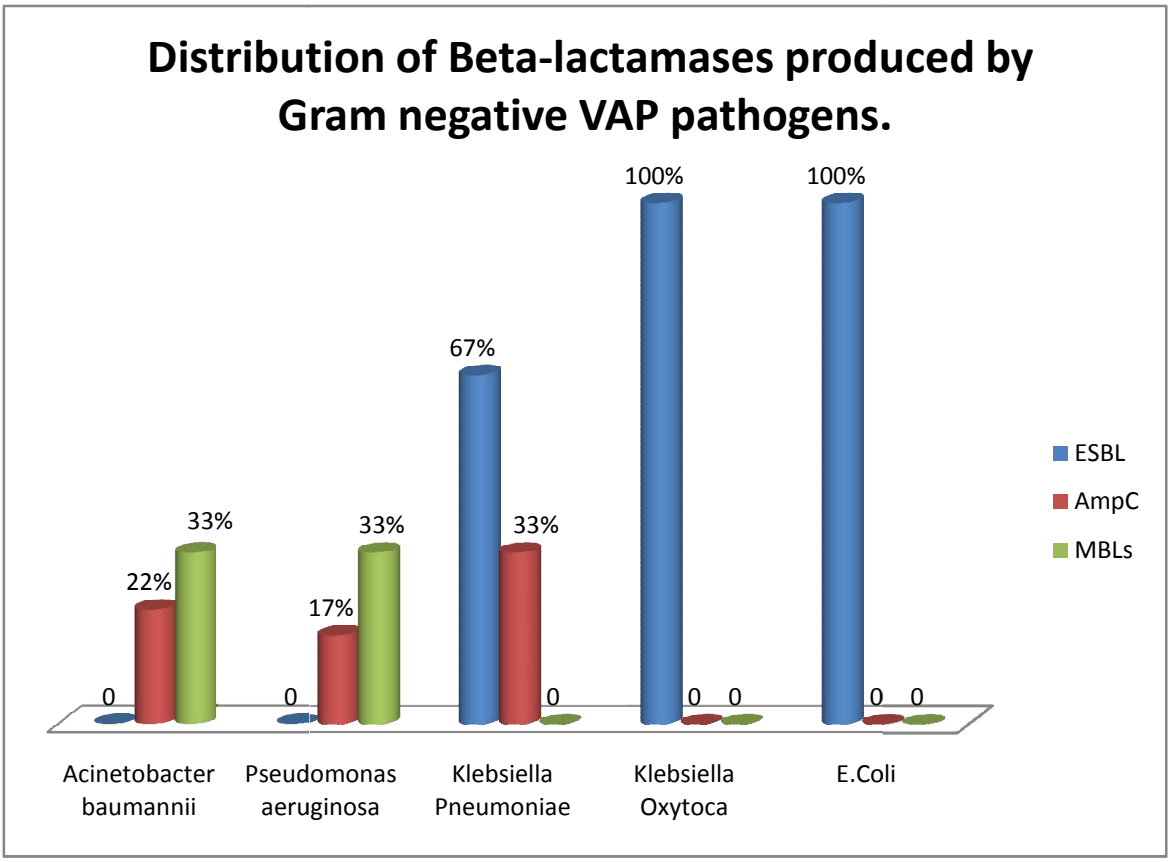
Antimicrobial sensitivity pattern of Enterobacteriaceae



The isolates of Enterobacteriacea were 67% sensitive to Amikacin83% to Piperazilin-tazobactum.All were sensitive to carbapenems.

Table20 Distribution of Beta-lactamases produced by Gram negative VAP pathogens.

Organism	**Total**	**ESBL**	**AmpC**	**MBLs**
Acinetobacterbaumannii	9	-	2(22%)	3(33%)
Pseudomonas aeruginosa	6	-	1(17%)	2(33%)
KlebsiellaPneumoniae	3	2(67%)	1(33%)	-
KlebsiellaOxytoca	2	2(100%)	-	-
E.Coli	1	1(100%)	-	-
Total	21	5	4	5
P value		0.007 Significant	0.007 Significant	0.001 Significant



Gram negative isolates among VAP pathogens were tested for the production of various beta lactamases, showed ESBL(Extended spectrum beta lactamase) production in 83% isolates of Enterobacteriaceae. ESBL was produced by 100% of E. coli, 67% of *K. pneumoniae,* 100% of *klebsiellaoxytoca.*

AmpC beta lactamases were produced by 33% of *klebsiellapneumonia* isolates, 22% of *Acinetobacterbaumannii*,17% of *Pseudomonas aeruginosa*.

MBL(Metallobeta lactamases) were produced by 33% of*Acinetobacterbaumannii* and 33% of *pseudomonas aeruginosa.*

Table 21:Antimicrobial sensitivity pattern of Staphylococcus aureus.

	Penicillin	Erythromycin	Ciprofloxacin	Cotrimoxazole	Amikacin	Gentamicin	Cefoxitin	Tetracyclin	Chloramphenicol
MRSA (2)	S	S	S	S	S	S	S	S	S
	0%	0%	50% (1)	0%	50% (1)	0%	0%	50% (1)	50% (1)

MRSA(Methicillin resistant Staphylococcus aureus) isolates were tested for Vancomycin MIC using Macro broth dilution method and E (Epsilometer) test method.

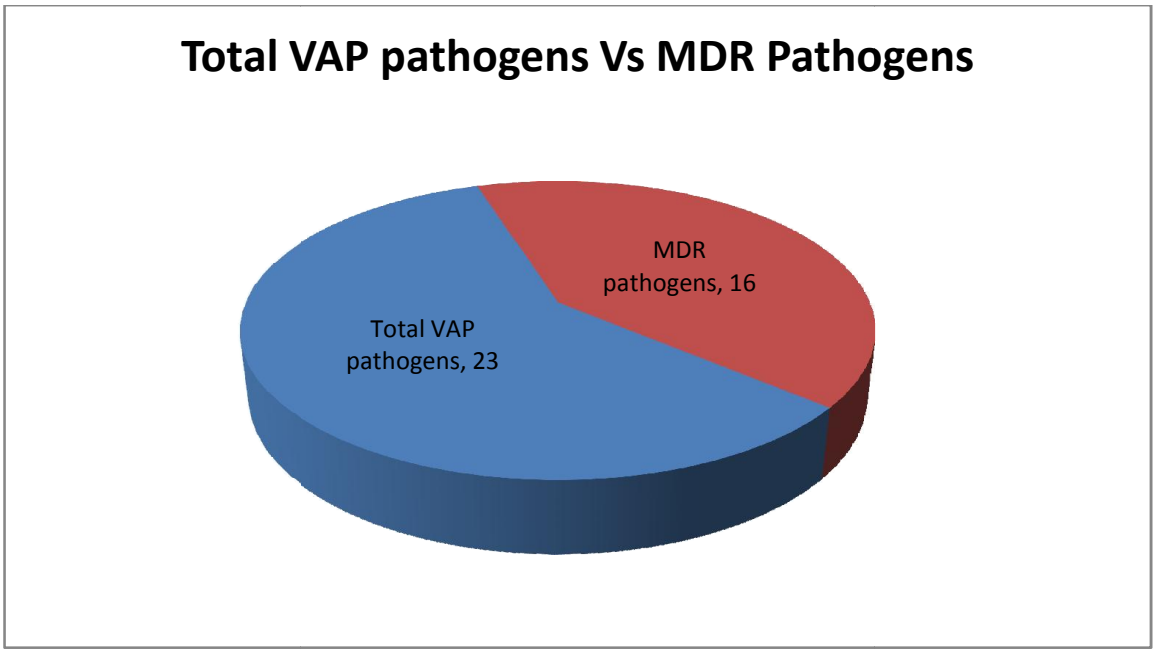
Table 22.Minimum Inhibitory Concentration of Vancomycin for MRSA isolates.

MRSA isolate	Vancomycin MIC Valuein µg/ml		Interpretation
	Macrobroth dilution method	E test method	
1	0.50	0.75	Sensitive
2	0.25	0.25	Sensitive

Both the MRSA isolates were sensitive to Vancomycin with an MIC value of less than 2µg/ml.

Table 23 Correlation of duration of mechanical ventilation,Onset of VAP with multi drug resistant organisms.

Duration of mechanical ventilation	Onset of VAP	Total VAP pathogens. N=23.	MDR pathogens N=16.	percentage	P value
≤ 4days	Early	8	5	63%	0.134 NS
≥ 5days	Late	15	11	73%	



Out of 23 VAP pathogens ,16 were multidrug resistant pathogens.

Distribution of MDR pathogens among VAP pathogens.(N=16)

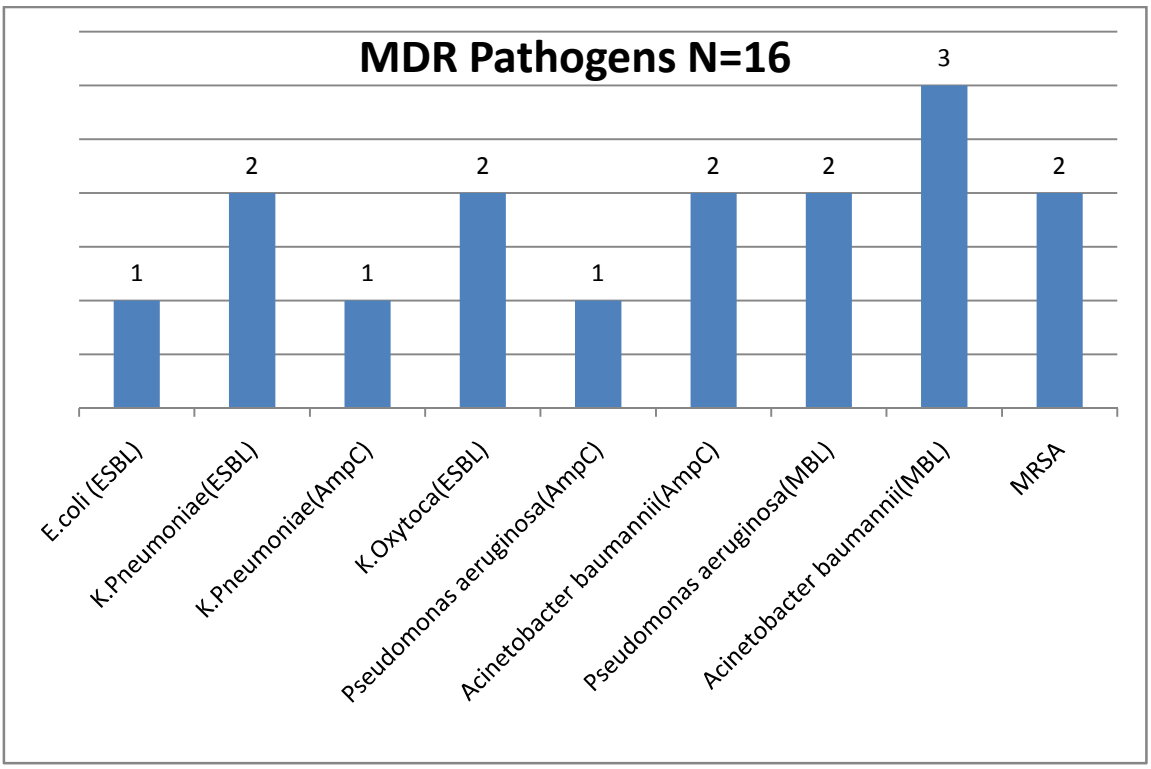


Table24 Clinical Outcome among patients with Suspected VAP.(N=100).

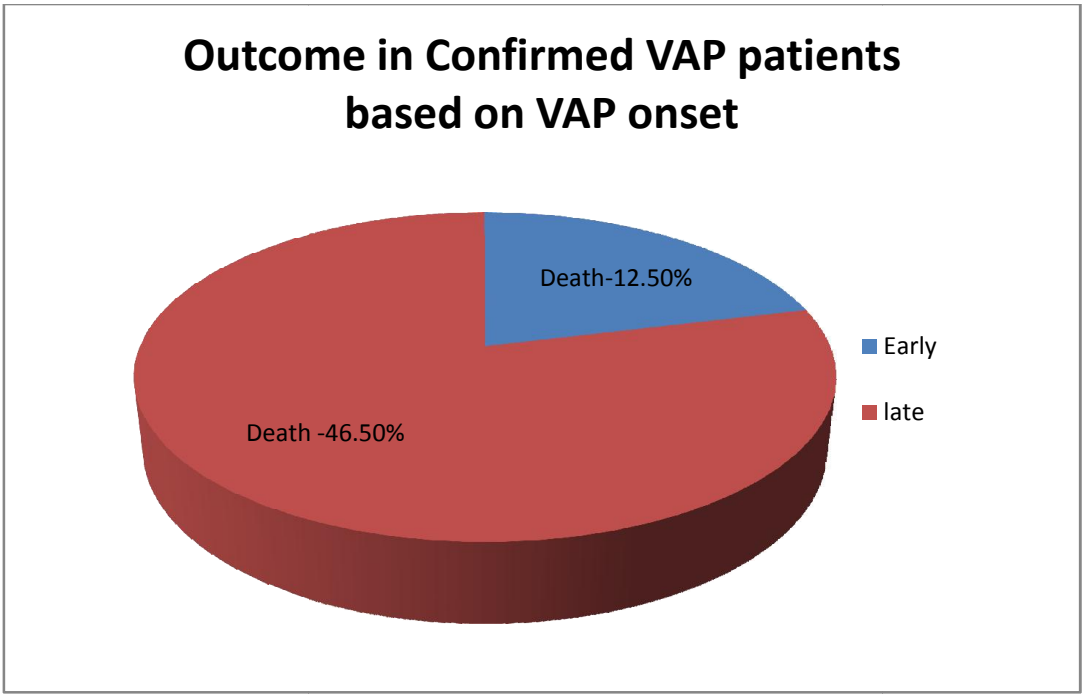
Clinical Outcome	Suspected VAP Patients(n=100)		Total	P value
	Confirmed VAP(n=23)	No VAP (n=77)		
Died	8(35%)	11(14%)	19	0.028 Significant
Discharged	15(65%)	66(86%)	81	

The outcome of VAP was considered in terms of death and discharge.

Out of 23 confirmed VAP patients ,8(35%) patients died.Hence mortality rate is 35% among the VAP patients.

Table 25 Clinical Outcome in confirmed VAP patients with respect to VAP onset.(n=23)

Outcome / Onset	Death	Discharged
Early (8)	1(12.5%)	7(87.5%)
Late (15)	7(46.5%)	8(53.5%)



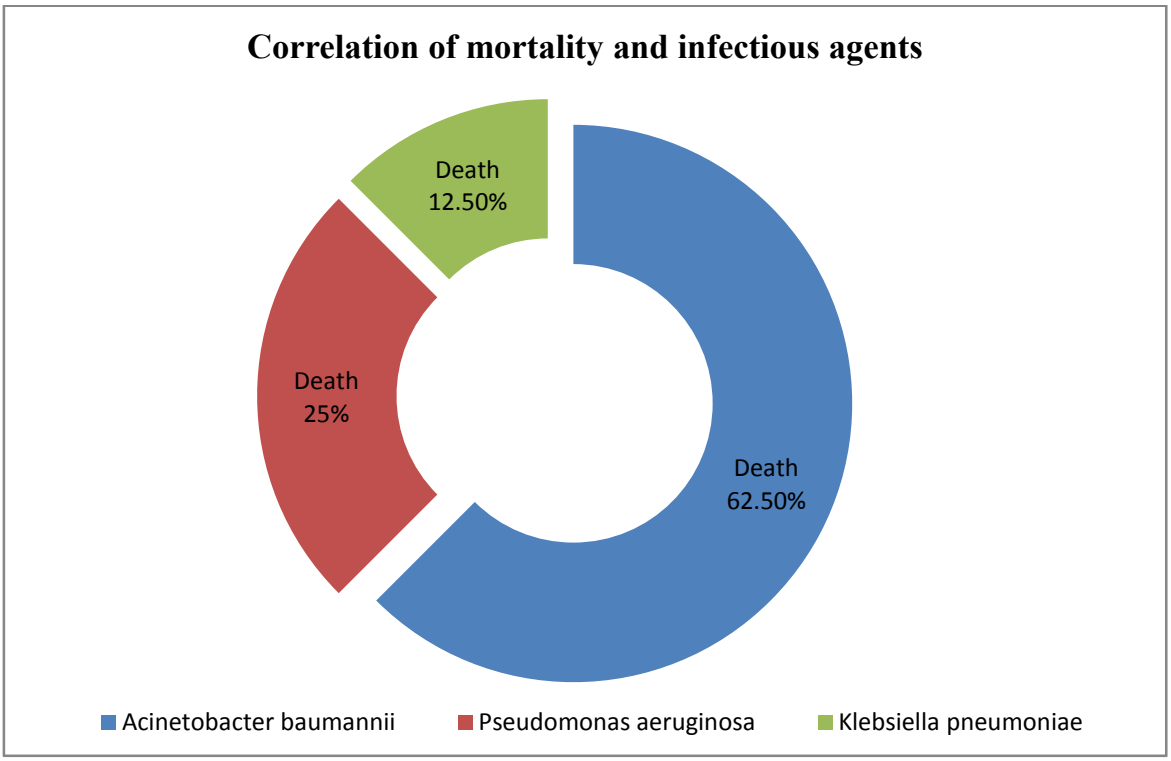
Themortality in late onset VAP was significantly high at 46.5% with the death of 7 patients out of 15 during the course of their illness.However mortality was relatively low at 12.5%in early onset VAP with the death of 1 patient out of 8 during the course of their illness. It was observed that more number of deaths occured in late onset VAP.

Table26Correlation of mortality and infectious agents among VAP patients(N=23)

Sr.no.	Organism	VAP Death N=8	Percentage	No.MDR Pathogen	Percentage Of MDR Pathogen
1	*Acinetobacterbaumannii*	5	62.5% (5/8)	4	80% (4/5)
2	*Pseudomonas aeruginosa.*	2	25% (2/8)	1	50% (1/2)
3	*Klebsiellapneumoniae*	1	12.5% (1/8)	1	100% (1/1)

The majority(62.5%) of patients died among confirmed VAP cases were due to *Acinetobactorbaumannii* infection .The next most lethal organism was *pseudomonas aeruginosa* with a mortality rate of 25% followed by *klebsiellapneumoniae* 12.5%.

Correlation of mortality and infectious agents:



The majority(62.5%) of patients died among confirmed VAP cases were due to *Acinetobactorbaumannii* infection.

DISCUSSION

This cross sectional study was done at the Institute of Microbiology, in association with the Institute of Internal Medicine, at the Rajiv Gandhi Government General Hospital, Chennai .

A total of 100 patients with suspected Ventilator associated pneumonia who satisfied the inclusion criteria were included in this study of which 67 were males and 33 were females with majority of the patients belong to the age group of less than 30yrs(25%) followed by 51-60yrs.(22%) [Table 1]

The clinical spectrum of study population indicates that the maximum number of cases enrolled in the study were of poisoning (33%) followed by Cardiovascular diseases(17%) [Table 2]

The American Thoracic Society guidelines suggests that Endotracheal aspirates and Bronchioalveolar lavage can provide more representative samples than the Protected specimen brush (PSB) which sample only a single bronchial segment.(2)

The samples obtained from the study population were Endotracheal aspirates,Bronchioalveolar lavage and Blood.[Table 3]

Pugin et al proposed Clinical Pulmonary Infection Score(CPIS) to improve the specificity of clinical diagnosis based on clinical,radiological,physiological and microbiological data in to a single numerical result.CPIS score of more than 6 was associated with a sensitivity of 93% and a specificity of 100% for the diagnosis of pneumonia.(31,37)In this study,23% of patients had CPIS score more than 6.

The American Thoracic Society guidelines states that a reliable tracheal aspirate Gram stain can be used to direct initial empiric antimicrobial therapy and may increase the diagnostic value of the CPIS.(2).

In this study,83% of patients with a significant quantitative culture($\geq 10^5$cfu/ml for ETA) had pus cells of more than 10/HPF with one or more bacteria per oil immersion field but none of the patients with a negative direct smear had a significant quantitative culture.[Table4,6]

Hence the presence of >10 pus cells /HPF with ≥1 bacteria per oil immersion field is an useful method for diagnosing VAP presumptively.

Those patients with CPIS more than 6 and quantitative culture of the Endotracheal aspirates $\geq 10^5$cfu/ml and Bronchioalveolar lavage $\geq 10^4$cfu/ml were confirmed with the diagnosis of VAP.[Table 5]

Based on this, 23% of patients in this study were confirmed with the diagnosis of VAP.[Table 7]

Various studies have reported the frequency of VAP ranging from 18% by Joseph et al to 57.14 % by Ranjan et al as shown below.

Frequency of VAP (comparison with other studies)

STUDY	YEAR	VAP Rates in percentage(%)
Agrawal et al (25)	2006	23
Joseph et al (18)	2009	18
Mukhopadhyay et al (27)	2010	42
Reena et al (28)	2011	27.22
Ranjan et al(70)	2014	57.14
Current study	2015	23

In this study,device associated incidence rate is 16 per 1000 ventilator days [Table 8] which is similar to the study by joseph et al from pondicherry where the incidence is 15.87per 1000 ventilator days in CCU(Critical Care Unit) and 30.67per 1000 ventilator days in MICU.Ranjan et al(70) from Madhya Pradesh reported the incidence of VAP as 31.7 per 1000 ventilator days whereas Singh et al(74) from Gujarat reported the incidence as 21.92 per 1000 ventilator days

The rates of VAP in surgical ICU were higher than in medical ICU's,depending on the difference in patient population,the proportion of patients that needed

mechanical ventilation and the duration of ventilation.The device associated incidence rate varies from 13.2-51 per 1000 ventilator days(12).

In this study VAP was most seen in age group of 51- 60 years[Table 9].It is correlating with a study by Dey et al(16), in that the most common age group to acquire VAP was between 46-60 years.The mean age for developing VAP was 45 years in study by Mukhopadhyay et al(27).In this study the mean age for developing VAP was 50 years.

Of the 23 patients who developed VAP in this study 74% were male and 26% were female which is similar to a study conducted by EleniApostolopoulou et al(12) 71% were male and 29% were female and also a study conducted in India by Joseph et al(18) reported that 66.7 % were male and 33.3 % were female.

In the present study,VAP was common among patients with Organophophorouspoisoning(30%)[Table10]. Similar study by Panwar et al(67) showed VAP associated predominantly with poisoning cases.Poisoning cases are subjected to gastric lavage prior to admission.These patients develop severe respiratory disease and therefore an increased need for mechanical ventilation.The pulmonary symptoms might be due to aspiration as a result of induced vomiting and lavage. Other conditions which required prolonged admission and mechanical ventilation like head injury,sepsis and intra abdominal diseases also developed VAP, which is in concurrence with other studies by Niederman et al(3) and Apostopoulou et al(12).

Out of the 23 cases of VAP, 34.8% were categorised as early-onset and 65.2% were late-onset VAP[Table 11]. Similar results were obtained by Mukhopadhyay et al(27) with38% being early-onset VAP and 62% late-onset VAP.The categorization of VAPis important for starting initial empiric antibiotic therapy.The late onset VAP is commonly associated with MDR pathogens,hence should be treated with broad spectrum antibiotics.(2)

In the current study,among the various risk factorsanalysed,reintubation(71%)(P value 0.025),Prior antibiotics(61.5%)(P value 0.023), and emergency intubation(60%) (P value 0.045),were significantly associated with VAP[Table 12].Similarly Agrawal et al(25) had reported an increased risk of VAP in patients who underwent re-intubation.Awarenessof these risk factors may help in identifying patients at increased risk for VAP and guide inimplementation of appropriate preventive measures during management.

In this study,it was observed that,hospitalization of 5 days or more is associated with the development of VAP with MDR pathogens like *Acinetobacterbaumanni* and *Pseudomonas aeruginosa* which is similar to a study by Ranjan et al(70).

In the present study, Gram negative bacilli were the predominant pathogens(91%) followed by Gram positive cocci(8%)[Table 13] similar to a study by Rajesh chawla et al(6) who reported that 87% of patients with VAP were infected with Gram negative bacilli.Among the Gram negative bacilli,non fermenters were the predominant pathogens causing VAP in our IMCU.

In the present study,*Acinetobacterbaumannii*(39%) followed by *pseudomonas aeruginosa(*26%) was found to be the most commonly isolated pathogens in VAP patients and also they are associated with late onset VAP ,similar to a study by craven et al(13).The common organism causing early onset VAP were from the group of Enterobacteriaceae like *Klebsiella pneumonia*(25%),*Klebsiellaoxytoca*(25%)and *E.coli*(12.5%).[Table 14,15]In a study by Dey et al (16) from Manipal,the commonest organismcausing both early and late onset VAPwas*Acinetobacter species* (48.94%) followed by *P.aeruginosa* (25.53%).

Hence,the knowledge of difference in microorganisms causing VAP in different ICU settings will guide the prescription of appropriate empirical antibiotics and treatment of the infection adequately.

Acinetobacterspecies(30*%),klebsiella pneumonia*(23%) and *pseudomonas aeruginosa* (18%)were the common organisms colonizing the respiratory tract of the patients on mechanical ventilation in this study[Table 16].The knowledge about the colonizers is important as reported by Alp et al (31)that the aspiration of colonizers on the oropharynx and those on the gastrointestinal tract is the main route for the development of Ventilator Associated Pneumonia.

Acinetobacter species are particularly important in causing nosocomial outbreaks and readily spread from one patient to another. This appears to be due to their ability to survive on the hands of health care workers and inanimate environmental surfaces and their intrinsic resistance to many common antibiotics rather than any potent virulence factors aimed at host defenses.

In thisstudy,out of 23 VAP patientsblood culture was positive in 8(35%) patients.Among the 8 patients,identical pathogens were isolated from both blood culture and respiratory sample in 5 patients(22%).[Table17] which is similar to a study byLuna et al(73)Antimicrobial susceptibility pattern of the isolates obtained in the present study showed that 70% of these VAP pathogens were resistant to commonly used drugs which is similar to a study conducted by Joseph et al(9) where 78.7% of VAP pathogens were multi drug resistant.

Acinetobacterbaumannii,the predominant pathogen isolated in this study showed decreased susceptibility to first line drugs like Ceftazidime(11%), quinolones(ciprofloxacin)(33%), and Amikacin(44%).They were 67% sensitive to carbapenems and 44% sensitive to Piperacillintazobactum.[Table18]

In *Pseudomonas aeruginosa*,67% isolates were sensitive to amikacin,67% to carbapenems,50% to Piperazilintazobactum.Similar results were obtained in studies conducted by Joseph et al(9) and Dey et al(16).

All the Nonfermentors showing resistance to carbapenems by Kirby Bauer disc diffusion method were further tested for MIC of meropenem by macrobroth dilution method.There was a good agreement between Kirby Bauer disc diffusion method and MIC in determining the susceptibility of non fermenters to meropenem in our study[Table 19],though Sinha et al(72) had reported discordant results between Kirby Bauer disc diffusion method and MIC determination by broth microdilution method with 18 out of 21 isolates resistant by disc diffusion method being sensitive to micro broth dilution method.

The isolates of *Enterobacteriaceae* were 67% sensitive to Amikacin ,33% sensitive to quinolones and 83% sensitive to Piperazilin-tazobactum.All isolates were sensitive to carbapenems.

The members of*Enterobacteriaceae*isolated in this study showed a high level of resistance(100%)to Extended spectrum cephalosporins. Emergence of Extended spectrum beta lactamases (ESBLs) and AmpCbetalactamasesin a hospital set up are of increasing concern.

In this study, ESBLproduction was observed in 67% of *K.pneumoniae*,100% of *Escherichia coli* and 100% of *Klebsiellaoxytoca*.[Table 20]In a study by Dey et al(16),100% of *K.pneumoniae* and 80% of *Escherichia coli* produced ESBLs.

Although there is nocurrent(Clinical Laboratory Standards institute) CLSI guidelines for detection of AmpC beta lactamases, the present study incorporated the AmpC-disc method suggested by Singhal et al(65) to detect AmpC beta lactamases.17% of *Pseudomonas aeruginosa*,22% of *Acinetobacterbaumannii,*and 33% of *Klebsiellapneumoniae*, have shown production of AmpC beta lactamases in our study ,similar to a study by Dey et al(16).

In this study ,33% of *Acinetobacterbaumannii* and 33% of *Pseudomonas aeruginosa* were metallobetalactamase enzyme producing strains detected by imipenem EDTA combined disc method[Table 20] whereas Hans et al(75) from U.P reported MBL production by 47.05% of *Acinetobacterbaumannii*. Dey et al (16) from Manipal reported MBL production in 21.74% of *Acinetobacterbaumannii* and 50% of *Pseudomonas aeruginosa.*

In this study 2 isolates of *Staphylococcus aureus* were isolated,of which both were *Methicillin resistantStaphylococcusaureus* and is associated with late-onset VAP.These isolates showed 50%sensitivity to quinolones and to Amikacin[Table 21].

All isolates were sensitive to Vancomycin which was detected by Macrobroth dilution method and E test method[Table 22].Whereas otherstudies have shownthat*Staphylococcusaureus* (methicillin sensitive or resistant) to be a major causative agent of early-onset VAP(3,69) which is different from the present study.This indicates that the causative agents may vary in different ICUsettings.

In the present study, it was found that the mortality rate among the VAP patients was 35%[Table 24]. Similar findings were reported in studies undertaken by Panwar et al(67) and Mukhopadhyay et al(27) where mortality rates were found to be 37% and 61.9% respectively.

In this study ,it was seen that the mortality was significantly high in patients with late-onset VAP(46.5%)[Table 25] caused by multidrug resistant *Acinetobacterbaumannii*and *Pseudomonas aeruginosa* infection when compared to early onset VAP(12.5%).[Table 26].

SUMMARY

- This cross-sectional study was done by enrolling patients admitted to MICU and receiving mechanical ventilation for ≥48 hours with clinical and radiological parameters indicative of VAP
- A total of 100 patients with suspected Ventilator associated pneumonia who satisfied the inclusion criteria were included in this study of which 67 were males and 33 were females with majority of the patients belong to the age group of less than 30yrs(25%) followed by 51-60yrs.(22%) .
- The clinical spectrum of patients included in the study were poisoning (33%) followed by Cardiovascular diseases (17%) and Intraabdominal diseases (13%).
- Endotracheal aspirates /BAL and blood samples were collected from suspected VAP patients and quantitative cultures were done on respiratory samples.
- The presence of >10 pus cells /HPF with ≥1 bacteria per oil immersion field is an useful method for presumptive diagnosis of VAP.
- VAP was diagnosed using the CPIS score > 6 and quantitative culture of $\geq 10^5$CFU/ml for endotracheal aspirates and $\geq 10^4$CFU/ml for BAL samples.
- Out of 100 suspected VAP patients,23 were confirmed with the diagnosis of VAP.
- The VAP rate in our study was 16 per 1000 ventilator days.

- Ventilator associated pneumonia was preponderant in males, the common age group being 51- 60 years.
- VAP was common among patients with Organophophorouspoisoning(30%).
- Among the 23 confirmed VAP patients, 34.8% had early-onset VAP and 65.2% had late-onset VAP.
- Among the risk factorsanalysed,reintubation(71%),Prior antibiotics(61.5%) and emergency intubation(60%) were predominantly associated with VAP. Hospitalization of 5 days or more is associated with the development of VAP with Multi drug resistant pathogens.
- Among the 23 VAP pathogens,91% were Gram negative bacilli and 9% were Gram positive cocci.
- Non fermenters(65%) were the predominant pathogens among Gram negative bacilli causing VAP in our IMCU.
- *Acinetobacterbaumannii* (39%) followed by *P.aeruginosa* (26%) were the commonly isolated pathogens in VAP patients which were associated with late onset VAP.
- The common organisms causing early onset VAP were from the group of *Enterobacteriaceae* like *Klebsiellapneumonia*(25%) and *Klebsiellaoxytoca(*25%).
- Out of 23 VAP patients, blood culture was positive in 35% patients of which 22% patients showed positive growth in blood culture with the same organism isolated from respiratory sample.
- Antimicrobial susceptibility pattern of the VAP isolates revealed that 70% of the pathogens to be multi-drug resistant.

- *Acinetobacterbaumannii* showed decreasedsusceptibility to first line drugs like Ceftazidime(11%), quinolones(ciprofloxacin)(33%), and Amikacin(44%).They were 67% sensitive to carbapenems and 44% sensitive to Piperacillintazobactum.
- In *Pseudomonas aeruginosa*,67% isolates were sensitive to amikacin,61% to carbapenems,50% to Piperazilintazobactum.
- The isolates of *Enterobacteriaceae* were 67% sensitive to Amikacin ,33% sensitive to quinolones and 83% sensitive toPiperazilin-tazobactum.All isolates of Enterobacteriaceae were sensitive to carbapenems.
- *Staphylococcus aureus* isolated in our study showed 50% sensitivity to quinolones and to Amikacin.All isolates were sensitive to Vancomycin ,detected by Macrobroth dilution method and E test method.Both the isolates were *methicillin resistant Staphylococcus aureus* and were associated with late onset VAP.
- 67% of *K. pneumoniae* , 100% of *K.oxytoca* and *E. coli* produced extended spectrum beta lactamases (ESBLs). Metallo β-lactamases (MBLs) production was seen in 33% of *Pseudomonas aeruginosa* and 33% of *Acinetobacterbaumannii.*AmpC beta lactamase production was seen in 33% of*klebsiella pneumonia*,22% of *Acinetobacterbaumannii* and 17% of *pseudomonas aeruginosa.*
- The mortality rate in ventilator associated pneumonia was 35 % in this study. The rate was higher in patients with multidrug resistant organisms like *Acinetobacterbaumannii* and*Pseudomonas aeruginosa* infection.

CONCLUSIONS:

- Ventilator associated pneumonia is preponderant in males, the common age group being 51- 60 years.
- VAP was common among patients with Organophophorous poisoning.
- Direct gram stain was found to be useful predictor of VAP.
- The significant risk factors associated with the development of VAP were re-intubation,prior antibiotics and emergency intubation.
- Gram negative bacilli are the significant contributor to the development of VAP,among which nonfermentors like *Acinetobacterbaumannii* followed by *pseudomonas aeruginosa*were the commonest pathogens isolated.
- The causative organisms for early onset VAP is different from late onset VAP.Increase in association of MDR pathogens with late onset VAP indicates that appropriate broad spectrum antibiotics should be prescribed.
- MDR pathogens increase mortality in VAP patients due to the production of ESBLs,AmpCbetalactamases and metallobetalactamases.
- Thus this study gives a knowledge on the baseline VAP rate,risk factors, the causative organism and the prevailing drug susceptibility pattern of our Medical Intensive Care Unit,which will benefit in improving the active surveillance programme aimed towards an effective hospital infection control strategy.

APPENDIX I

ABBREVIATIONS

VAP -Ventilator-associated pneumonia.

HAP -Hospital Acquired Pneumonia.

ICU -Intensive Care Unit.

MICU -Medical Intensive Care Unit.

MV -Mechanical Ventilation.

MDR -Multi Drug Resistant.

ETA -Endotracheal Aspirate.

BAL -Bronchioalveolar Lavage.

PSB -Protected Specimen Brush.

ESBL -Extended Spectrum Beta lactamases.

MBL -Metallobeta lactamases.

MRSA -Methicillin resistant Staphylococcus aureus.

MSSA -Methicillin sensitive Staphylococcus aureus.

MIC -Minimum Inhibitory Concentration

CLSI -Clinical Laboratory Standards Institute.

ATCC -American Type Culture Collection.

IHI –Institute of Healthcare Improvement

APPENDIX II

A. STAINS AND REAGENTS

1. Gram staining

Methyl violet (2%)	l0g Methyl violet in 100ml absolute alcohol in 1 litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbol fuchsin 1%	Secondary stain.

2.10%KOH

Potassium hydroxide	10g
Glycerol	10ml
Distilled water	80ml

3.Lacto Phenol Cotton blue stain

Lactic acid	20ml
Phenol	20ml
Cotton blue(dye)	0.5g
Glycerol	40ml
Distilled water	20ml

B. MEDIA USED:

1. Mac Conkey agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1ltr
Agar	20g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	100ml

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix. Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

3. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	l0g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

4. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

5. Cation adjusted Mueller- Hinton Agar

Beef infusion	300ml
Caesein hydrolysate	17.5g
Starch	1.5g
Agar	l0g
Distilled water	7 Lltr

pH = 7.4

Sterilise by autoclaving at 121°C for 20 mins

C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1. Oxidase Reagent

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2. Catalase

3% hydrogen peroxide

3. Indole test

Kovac's reagent

Amyl or isoamyl alcohol 150ml Para dimethyl amino benzaldehyde lOg
Concentrated hydrochloric acid 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Christensen's Urease test medium

Peptone	lg
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	10ml
Sterile 20% urea solution	100ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20 g
Bromothymol blue 0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6. Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	lg
Lactose	10 g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
S odum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	12ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	l0mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with Durham's tube. Basal medium peptone water Sugar solutions:

Sugar	1ml
Dislilled water	100ml

pH = 7.6.

9. Mannitol motility medium

Agar	5g
Peptone	lg
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	1000ml
pH	7.2

10. Phenolphthalein diphosphate agar

- Sterilize a 1% aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4°C
- Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50°C and pour plates
- Grow the staphylococcus overnight at 37°C on the medium
- Invert the plate and pour a few drops of ammonia solution SG 0.88 into the lid
- Read as positive a culture whose colonies turn bright pink within a few minutes. The colour soon fades.

11. Potassium nitrate broth

Potassium nitrate (KNO_3)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes and autoclaved.

12. Phenyl alanine deaminase test

Yeast Extract	3g
Dl-Phenylalamine	2 g
Disodium hydrogen phosphate	1 g
Sodium Chloride	5 g
Agar	12g
Distilled water	1 lr
PH	7.4

Distributed in tubes and sterilized by autoclaving at 121° C for 1 5 minutes, allowed to solidify as long slopes.

13. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml
Sugar to be tested	20g
Water	1 litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1 litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3 consecutive days

ANNEXURE-I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. K.Vasanthi,
Postgraduate M.D.(Microbiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr.K.Vasanthi,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study on ventilator associated pneumonia with special reference to multi drug resistant pathogens in a tertiary care hospital". No.17102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr.C.Rajendran, M.D.,	: Chairperson
2.	Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3.	Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4.	Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5.	Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
6.	Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
7.	Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
8.	Thiru S.Rameshkumar, Administrative Officer	: Lay Person
9.	Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10.	Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee
MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE-II

PROFORMA

Name : IP no / Ward:

Age/Sex: Address: Occupation:

Mechanical ventilation: Started On- Duration-

Prior Antibiotic therapy: Recent change in antibiotic:

Presenting complaints:

Co-Morbid Conditions:

Past history:

Personal history:

Risk Factors:

Physical examination:

RS: Temp: RR:

CVS: BP: PULSE:

CNS: ABD:

Laboratory evaluation:

TC		Plasma glucose levels	
DC		Blood urea	
ESR		Sr. Creatinine	
Hb estimation		Arterial blood gas analysis	
Peripheral Smear		X-ray	
Liver function test		Others	

Microbiological investigation:

Sample collected: Endotracheal Aspirate / BAL fluid /Blood

Direct examination:

Gram'stain: KOH mount:

Bacterial Culture:

NAP:

MAC:

BAP/CAP:

Biochemical reactions:

Fungal culture: SDA with antibiotics.

Blood culture:

Isolate identified in Respiratory sample:

Isolate identified in blood sample:

Antibacterial susceptibility pattern:

Multi- drug resistance tests-

Antifungal susceptibility pattern:

Clinical pulmonary infection score (CPIS):

Temperature	≥38.5°C & ≤ 38.9°C	Point 1	
	>39°C or <36°C	Point 2	
Blood leucocyte count (cells/mm^3)	<4000 or >11000.	Point 1	
	+ >50% band forms	Point 2	
Oxygenation(mmHg) Pa02/Fio2.	<240 and no ARDS	Point 2	
Chest X-ray	No infiltrates	Point 0	
	Patchy or diffuse infiltrates	Point 1	
	Localised infiltrates	Point 2	
Tracheal secretions (subjective visual scale)	Mild/non purulent	Point 1	
	Purulent	Point 2	
Culture & Gram stain of endotracheal aspirate.	Moderate or heavy growth	Point 1	
	Same morphology on Gram stain	Point 2	

CPIS score: (CPIS score >6 → VAP)

Diagnosis:

Treatment:

Outcome:

ANNEXURE-III

PATIENT CONSENT FORM

Title of the study: “A study on ventilator associated pneumonia with special reference to multidrug resistant pathogens in a tertiary care hospital.”

Name : Date :

Age : IP No :

Sex : Project Patient No :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I hereby give my consent to be included as a participant in “A study on ventilator associated pneumonia with special reference to multidrug resistant pathogens in a tertiary care hospital” and I give consent to collect my lower respiratory samples (Endotracheal aspirate/BAL fluid) and Blood sample for further investigations.

I have read and understood this consent form and the information provided to me.

I have had the consent document explained to me.

I have been explained about the nature of the study.

I have been explained about my rights and responsibilities by the investigator.

I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.

I have been advised about the risks associated with my participation in this study.I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.I have not participated in any research study within the past _____ month(s).

I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.

I hereby give permission to the investigator to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

I have understood that my identity will be kept confidential if my data are publicly presented.I have had my questions answered to my satisfaction.

I have decided to be in the research study.I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For participants:

Name and signature / thumb impression of the participant (or legal representative if participant in competent/For age 10-17 yrs-Name& signature of the parent/guardian.)

Name _____

Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____

Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____

Signature_____

Date_____

Master Chart:

Sr.No	I.P.No	Age	Gender	Diagnosis	Risk Factors								CPIS Score	MV Duration (Days)	VAP	VAP Onset	Respiratory Sample	Quantitative Culture	Antimicrobial Susceptibility Pattern																	MBL	ESBL	AmpC	Blood Culture	Colonizer	Outcome
					Emergency Intubation	Tracheostomy	Impaired Conciousness	Re intubation	Stress ulcer prophylaxis	Prior antibiotics	Naso gastric tube	IV Sedation							Penicillin	Amikacin	Gentamycin	Ciprofloxacin	Cefotaxime	Ceftazidime	Ceftazidime&clavulanic ac	Cotrimoxazole	Erythromycin	Cefoxitin	Vancomycin	Tetracyclin	Chloramphenicol	Imipenem	Meropenem	Piperacillin-tazobactum						
1	132762	45	M	OPC Poisoning		+			+	+			7	7	P	Late	ETA	Pseudomonas aeruginosa	-	S	R	R	-	R	-	-	-	R	-	-	-	S	S	R	-	-	P	NG	-	Discharged
2	94766	20	F	Spastic Quadriparesis		+			+	+		+	8	11	P	Late	ETA	Acinetobacter baumannii	-	R	R	R	-	S	-	R	-	S	-	-	-	S	S	S	-	-	-	NG	Klebsiella Pneumoniae	Died
3	3921	74	M	CVA-Encephalopathy					+				4	3	N	-	ETA	-												-								NG	Pseudomonas aeruginosa	Discharged
4	2728	22	F	Tablet Poisoning					+	+			4	3	N	-	ETA & BAL	-																				NG	Acinetobacter baumannii (ETA & BAL)	Died
5	132844	21	M	OPC Poisoning							+		4	4	N	-	ETA	-																				NG	Escherichia coli	Discharged
6	252	35	M	OPC Poisoning		+	+			+			8	14	P	Late	ETA	Pseudomonas aeruginosa	-	S	S	R	-	S	-	-	-	S	-	-	-	S	S	S	-	-	-	Pseudomonas aeruginosa	Klebsiella Pneumoniae	Died
7	9480	80	F	CKD					+				3	4	N	-	ETA	-																				NG	NG	Discharged
8	10019	56	M	OPC Poisoning				+		+	+		9	3	P	Early	ETA	Klebsiella Pneumoniae	-	S	R	R	R	R	S	R	-	-	-	-	-	S	S	S	-	P	-	klebsiella pneumoniae	-	Discharged
9	667	67	M	Sepsis		+							3	10	N	-	ETA	-																				NG	Candida albicans	Discharged
10	1138	59	M	Metabolic-Encephalopathy		+	+			+			9	20	P	Late	ETA	Pseudomonas aeruginosa	-	R	R	R	-	R	-	-	-	R	-	-	-	R	R	R	P	-	-	NG	-	Died
11	8640	29	M	Acute Meningoencephalitis					+				4	9	N		ETA & BAL	-																				NG	Acinetobacter baumanni(ETA & BAL)	Died
12	8986	52	M	Sepsis							+		5	6	N	-	ETA	-																				NG	Pseudomonas fluorescence	Died
13	132256	45	M	CVA					+				3	4	N	-	ETA	-																				NG	Pseudomonas aeruginosa	Discharged
14	8802	21	F	Insectiside Poisoning		+				+			3	8	N	-	ETA	-																				Pseudomonas fluorescence	NG	Died
15	10806	55	M	Meningitis					+				3	4	N	-	ETA	-																				NG	NG	Discharged
16	111723	61	M	Peritonitis					+				3	8	N	-	ETA	-																				NG	NG	Discharged
17	11781	41	M	OPC Poisoning			+			+			4	5	N	-	ETA	-																				NG	Pseudomonas aeruginosa	Died
18	12143	35	F	Sepsis					+				4	4	N	-	ETA	-																				NG	NG	Discharged
19	11206	19	F	CKD		+				+			3	9	N	-	ETA	-																				NG	Candida albicans	Discharged
20	13465	27	M	Sepsis			+						4	3	N	-	ETA	-																				NG	Acinetobacter lwoffii	Discharged
21	11822	68	F	DM/HT/CAD					+				4	6	N	-	ETA	-																				NG	NG	Discharged
22	10738	65	F	CAD/CCF					+	+			5	4	N	-	ETA & BAL	-																				NG	Klebsiella Pneumoniae (ETA & BAL)	Died
23	13824	55	M	OPC Poisoning				+			+		9	3	P	Early	ETA	Klebsiella Oxytoca	-	R	R	S	R	R	S	R	-	-	-	-	-	S	S	S	-	P	-	KlebsiellaOxytoca	-	Discharged
24	13894	55	M	OPC Poisoning					+				3	3	N	-	ETA	-																				NG	NG	Discharged
25	12398	50	M	OPC Poisoning			+						3	7	N	-	ETA	-																				NG	NG	Discharged
26	11396	60	M	CAD	+					+		+	9	3	P	Early	ETA	baumannii	-	S	S	S	-	R	-	S	-	S	-	-	-	S	S	S	-	-	-	NG	-	Discharged
27	15934	32	M	OPC Poisoning							+		3	3	N	-	ETA	-																				NG	NG	Discharged
43	12770	30	M	OPC Poisoning			+			+			9	4	P	Early	ETA	Oxytoca	-	S	S	R	R	R	S	R	-	-	-	-	-	S	S	S	-	P	-	MSSA	-	Discharged
44	16886	50	F	Sepsis		+						+	9	7	P	Late	ETA	baumannii	-	R	R	R	-	R	-	R	-	S	-	-	-	S	S	S	-	-	-	NG	-	Discharged
45	16679	48	F	Liver Disease			+						2	3	N	-	ETA	-																				NG	NG	Discharged
46	13406	25	F	OPC Poisoning					+				4	3	N	-	ETA	-																				NG	NG	Discharged
47	11051	54	F	Insectiside Poisoning									2	3	N	-	ETA	-																				Pseudomonas aeruginosa	NG	Discharged
48	88826	82	M	Diabetic Nephropathy				+		+			7	6	P	Late	ETA & BAL	Acinetobacter baumannii(ETA&BAL)	-	R	R	R	-	R	-	R	-	R	-	-	-	R	R	R	P	-	-	NG	-	Died
49	4776	55	M	Sepsis	+								8	3	P	Early	ETA	aeruginosa	-	S	S	S	-	R			-	S	-	-	-	S	S	S	-	-	-	NG	-	Discharged
50	597	50	M	Head Injury					+				2	4	N	-	ETA	-																				NG	NG	Discharged
51	20541	60	F	Hemiparesis					+				2	3	N	-	ETA	-																				MRSA	NG	Discharged
52	20472	65	F	Head Injury					+	+		+	7	5	P	Late	ETA	baumannii	-	S	S	R	-	R	-	S	-	S	-	-	-	S	S	S	-	-	-	r baumanni	-	Discharged
53	20059	63	M	CAD	+								7	3	P	Early	ETA	aeruginosa	-	S	S	R	-	S	-	-	-	S	-	-	-	S	S	S	-	-	-	NG	-	Discharged
54	16456	25	M	Insectiside Poisoning									2	3	N	-	ETA	-																				NG	Acinetobacter baumanni	Discharged
55	22851	72	M	OPC Poisoning				+					4	5	N	-	ETA	-																				NG	Staphylococcus epidermidis	Discharged
56	15293	35	F	High Cord Compression							+		3	3	N	-	ETA & BAL	-																				NG	Klebsiella Pneumoniae (ETA & BAL)	Discharged
57	13460	70	M	Head Injury				+		+			8	5	P	Late	ETA	Acinetobacter baumannii	-	S	R	S	-	R	-	R	-	R	-	-	-	S	S	R	-	-	P	Klebsiella Pneumoniae	-	Discharged
58	10421	26	M	Sepsis							+		4	4	N	-	ETA	-																				NG	Acinetobacter baumanni	Discharged
59	13243	47	M	Acom Aneurysm							+		4	3	N	-	ETA	-																				NG	NG	Discharged
60	14310	25	F	OPC Poisoning			+			+			6	5	P	Late	ETA	Pseudomonas aeruginosa	-	R	R	R	-	R	-	-	-	R	-	-	-	R	R	R	P	-	-	Pseudomonas aeruginosa	-	Discharged

61	21795	68	M	Sepsis					+				2	2	N	-	ETA	-																				NG	Staphylococcus aureus(MSSA)	Discharged
62	21904	32	F	Sepsis					+				4	3	N	-	ETA	-																				NG	NG	Discharged
63	19316	55	M	Hemiplegia							+		4	4	N	-	ETA	-																				NG	Klebsiella Pneumoniae	Discharged
64	20603	40	M	Acom Aneurysm								+	4	5	N	-	ETA	-																				NG	Klebsiella Oxytoca	Discharged
65	12453	60	M	CAD			+			+			4	4	N	-	ETA	-																				MSSA	Klebsiella Pneumoniae	Discharged
66	23395	45	F	OPC Poisoning	+					+			4	2	N	-	ETA	-																				NG	NG	Discharged
67	12342	27	M	OPC Poisoning					+				3	3	N	-	ETA	-																				NG	Pseudomonas aeruginosa	Discharged
68	13426	25	F	OPC Poisoning					+				4	4	N	-	ETA	-																				NG	Staphylococcus epidermidis	Discharged
69	23712	45	F	Head Injury			+						3	3	N	-	ETA	-																				NG	NG	Discharged
70	27829	55	M	CAD		+							3	8	N	-	ETA	-																				NG	NG	Discharged
71	23561	56	M	Central Venous Thrombosis		+							3	7	N	-	ETA	-																				NG	Staphylococcus aureus(MSSA)	Discharged
72	25590	19	F	OPC Poisoning								+	3	4	N	-	ETA	-																				NG	NG	Discharged
73	23960	23	M	OPC Poisoning			+			+			3	3	N	-	ETA	-																				Klebsiella Pneumoniae	NG	Discharged
74	24565	58	F	GBS					+				3	5	N	-	ETA	-																				NG	Acinetobacter lwoffii	Discharged
75	25352	51	M	Neurotoxic Snakebite			+						4	4	N	-	ETA	-																				NG	NG	Discharged
76	22234	35	F	MND		+							5	8	N	-	ETA	-																				NG	Acinetobacter baumanni	Died
77	248177	72	M	CKD					+				5	5	N	-	ETA	-																				NG	Klebsiella Pneumoniae	Discharged
78	23265	40	M	Neurotoxic Snakebite		+							4	8	N	-	ETA	-																				NG	NG	Discharged
79	18900	35	M	Head Injury					+	+		+	9	5	P	Late	ETA	aureus(MRSA)	R	S	R	S	-	-	-	R	R	R	S	R	S	-	-	-	-			NG	-	Discharged
80	27631	45	M	GBS		+							4	13	N	-	ETA	-																				Klebsiella Pneumoniae	NG	Died
81	27466	25	M	OPC Poisoning					+				4	6	N	-	ETA	-																				NG	Staphylococcus aureus(MSSA)	Discharged
82	26286	45	F	Meningitis		+				+		+	9	10	P	Late	ETA	Acinetobacter baumannii	-	R	R	R	-	R	-	R	-	R	-	-	-	R	R	R	P	-	-	MSSA	-	Died
83	29049	74	M	CVA-Encephalopathy		+	+			+			9	12	P	Late	ETA & BAL	Klebsiella Pneumoniae (ETA & BAL)	-	R	R	R	R	R	R	R	-	R	-	-	-	S	S	R	-		P	NG	-	Discharged
84	25598	65	M	CVA-Encephalopathy								+	5	4	N	-	ETA	-																				NG	Acinetobacter baumanni	Discharged
85	29139	18	M	Hemiparesis			+						4	7	N	-	ETA	-																				NG	Klebsiella Oxytoca	Discharged
86	44174	25	F	OPC Poisoning							+		4	4	N	-	ETA	-																				NG	Pseudomonas aeruginosa	Discharged
87	45230	32	M	OPC Poisoning						+		+	4	3	N	-	ETA	-																				NG	NG	Discharged
88	43666	52	M	Hemiparesis			+						5	6	N	-	ETA	-																				NG	Acinetobacter baumannii	Discharged
89	24877	72	M	CKD					+	+			4	6	N	-	ETA	-																				MSSA	Acinetobacter lwoffii	Died
90	44055	45	M	CVA-Encephalopathy							+		3	3	N	-	ETA	-																				NG	NG	Discharged
91	34243	52	M	OPC Poisoning							+		3	5	N	-	ETA	-																				NG	Acinetobacter baumannii	Discharged
92	34249	26	M	OPC Poisoning		+							4	12	N	-	ETA & BAL	-																				NG	Pseudomonas aeruginosa (ETA & BAL)	Discharged
93	35208	70	F	Diabetic Nephropathy					+				3	4	N	-	ETA & BAL	-																				NG	Klebsiella Pneumoniae (ETA & BAL)	Discharged
94	40544	50	M	MND		+				+			2	15	N	-	ETA	-																				Klebsiella Pneumoniae	NG	Discharged
95	42340	68	M	Diabetic Nephropathy		+							4	12	N	-	ETA	-																				NG	Acinetobacter baumannii	Died
96	50603	50	M	GBS					+				4	7	N	-	ETA	-																				NG	NG	Discharged
97	30010	56	M	Hemiparesis							+		3	3	N	-	ETA	-																				NG	NG	Discharged
98	31020	65	M	CVA-Encephalopathy						+		+	4	4	N	-	ETA	-																				Klebsiella Pneumoniae	Klebsiella Pneumoniae	Discharged
99	31142	40	F	CKD			+						3	3	N	-	ETA	-																				NG	NG	Discharged
100	62241	50	M	OPC Poisoning				+					3	6	N	-	ETA	-																				NG	Pseudomonas aeruginosa	Discharged

KEY TO MASTER CHART

M Male

F Female

P Present.

N Not present.

MND Motor Neuron Disease.

CVD Cardiovascular disease.

OPC Organophosphorous compound.

CKD Chronic Kidney Disease.

AK -Amikacin, COT-Cotrimoxazole,

CIP -ciprofloxacin, CTX-cefotaxime,

CAZ -ceftazidime,

CAC -Ceftazidime & clavulanic acid,

CX -cefoxitin, GM-Gentamicin,

IMP -Imipenem, MER-Meropenem,

PT -Piperacillin tazobactum.

P -Penicillin, TET-Tetracyclin,

CK -Chloremphenicol.

R Resistant

S Sensitive

CPIS Clinical Pulmonary Infection Score.

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